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Life Systems, Inc., Cleveland, OH

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on trichloroethylene This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of trichloroethylene
- Toxicokinetics and human exposure to trichloroethylene
- Health Effects of trichloroethylene in humans and animals
- Mechanisms of toxicity of trichloroethylene
- Quantification of toxicological effects of trichloroethylene

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by

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for

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PREFACE

The objective of this document is to assess the health effect information on the contaminant, trichloroethylene, in drinking water and to recommend a maximum contaminant level. To achieve this objective, data on pharmacokinetics, assessment of human exposure, acute and chronic health effects in animals and human health effects, including epidemiology and mechanisms of toxicity, were evaluated. Only the reports which were considered pertinent for the derivation of the maximum contaminant level are cited in the document. Particular attention was paid toward the utilization of primary references for the assessment of health effect. Secondary references were used rarely. For comparison, standards and criteria developed by other organizations are included and discussed in Section IX, Quantification of Toxicological Effects.

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I. SUMMARY

Trichloroethylene (TCE), $Cl_2C = CHCl$ is a colorless solvent. It has been used as a degreasing solvent in metal industries and in dry-cleaning shops and as an inhalation anesthetic during certain short-term surgical procedures.

The widespread use of TCE has resulted in its detection in air, food and human tissues. It has also been detected in the surface water and groundwater supplies of several states across the United States.

On ingestion from either food or drinking water, TCE is expected to be readily absorbed from the gastrointestinal (G.I.) tract and enter the bloodstream. About 95% to 98% of a single oral dose of TCE was absorbed from the G.I. tract in rats and mice. After entering the bloodstream, it distributes into various tissues and organs. It has been demonstrated that the extent of distribution depends largely on the fat content of the tissues. Trichloroethylene has been shown to be transported across placental barriers in pregnant women.

Trichloroethylene is metabolized to monochloroacetic acid, dichloroacetic acid, trichloroacetaldehyde (chloral), trichloroethanol, trichloroacetic acid, trichloroethanol glucuronide, oxalic acid and N-hydroxyacetyl-aminoethanol. Studies have provided evidence that TCE may be metabolized to to the above-mentioned metabolites via an epoxide intermediate, 2,2,3-trichloroxirane. This intermediate may be responsible for the mutagenic and carcinogenic potential of TCE; however, interaction of the epoxide with the nuclear material, a step towards carcinogenesis, has not been studied. It is noteworthy that TCE-epoxide has been demonstrated to bind with tissue macromolecules. This is characteristic of other carcinogens.

Among the acute and chronic adverse effects in animals, hepatotoxicity appeared to be of greatest importance. Nephrotoxic effects have also been reported in rats and mice. At very high dosages, TCE depressed myocardial contractility. High oral doses of TCE impaired copulatory behavior in male mice. Female fertility and estrus cycle were not affected by high oral TCE doses. Neonatal mortality in offspring from TCE exposed dams was significantly increased although no malformations were evident. Reports on the teratogenic effects of TCE indicated no teratogenic abnormalities in mice or rats.

Trichloroethylene was mutagenic in bacterial test systems utilizing liver microsomal fractions for activation. Orally administered trichloroethylene was found to be carcinogenic in $B_6C_3F_1$ strain mice; however, it was not carcinogenic in Osborne-Mendel rats. The validity of the study was questioned because carcinogenic impurities were detected in the test compound. The NCI repeated the experiment with pure TCE and again found TCE to be carcinogenic in $B_6C_3F_1$ mice.

There was no indication of tumorigenic potential of amine-based stabilized TCE in ICR/HA mice in a chronic oral study. The significance of this study is uncertain since dosing was intermittently interrupted and dose levels were decreased due to non-specific toxicity and poor condition of the animals. Another gavage study in ICR/HA Swiss mice found no increased incidence of tumors in the stomach, kidney or liver. Trichloroethylene was not carcinogenic in ICR/HA Swiss mice when administered by subcutaneous injection or skin painting. Inhalation studies found an increased incidence of malignant lymphomas in female NMRI mice and pulmonary adenocarcinomas in female ICR mice; the tumor incidence in WIST rats, Sprague-Dawley rats and Syrian hamsters was not affected by TCE inhalation.

Central nervous system, cardiotoxic, hepatotoxic and nephrotoxic effects have been reported in humans exposed to TCE in the workplace, through inhalation abuse and by accidental ingestion. The reports are clouded by the fact that the subjects were exposed to either the contaminated TCE and/or its decomposition products. Some of the effects, however, have been observed in animals under experimentally controlled conditions with reasonably pure TCE. Furthermore, dose-response relationships have been observed.

Based on the mechanism of toxicity, specifically, mutagenesis and carcinogenesis, TCE has been shown to be potentially carcinogenic. Trichloroethylene has been reported to bind with mouse liver DNA in an *in vivo* experiment. Covalent binding of calf thymus DNA with TCE in an *in vitro* experiment provided further support to the carcinogenic potential of TCE.

The National Academy of Sciences (NAS) and EPA's Carcinogen Assessment Group (CAG) have calculated projected incremental excess cancer risks associated with the consumption of a specific chemical via drinking water by mathematical extrapolation from high-dose animal studies (Table I-1). Using the risk estimates generated by the NAS (1977), where the multi-stage model was utilized, the range of TCE concentrations which would nominally increase the risk of one excess cancer per million (10^6), per hundred thousand (10^5) and per ten thousand (10^4) people over a 70-year lifetime was computed, assuming daily consumption at the stated exposure level. From the NAS model, it was estimated that, at the 95% confidence limit, consuming 2 L of water having TCE concentrations of 450 ug/L, 45/L or 4.5 ug/L per day over a lifetime would increase the risk of one excess cancer per 10^4, 10^5 or 10^6 people exposed, respectively. Using the revised CAG approach, and thus the "improved" multi-stage model, it can be estimated that, at the 95% confidence limit, consuming 2 L of water having TCE concentrations of 280 ug/L, 28 ug/L or 2.8 ug/L per day over a lifetime would increase the risk of one excess cancer per 10^4, 10^5 or 10^6 people exposed, respectively. The numerical

Table I-1 Drinking Water Concentrations and Associated Cancer Risks

	Range of Concentrations (ug/L)[a]		
Excess lifetime cancer risk	CAG (95% confidence limit)	NAS (95% confidence limit)	NAS (point estimate)
10^{-4}	280	450	1,400-450
10^{-5}	28	45	140-45
10^{-6}	2.8	4.5	14-4.5

[a] Assuming 2 L of water consumed per day.

differences observed after utilizing the NAS and the CAG risk estimates were partly due to the fact that the dose extrapolation model used by the two groups was similar, but not identical. The NAS used the multi-stage model, whereas the CAG used the "improved" version of the multi-stage model recently discussed by Crump (USEPA 1980). In addition, the selection of the data and other parameters in each model would also result in some differences.

II. INTRODUCTION

Trichloroethylene (1,1,2-trichloroethylene; TCE), C_2HCl_3, is a clear colorless liquid that is used mainly as a degreasing solvent in metal industries. It is also used as a household and industrial dry-cleaning solvent, an extractive solvent in foods and an inhalation anesthetic during certain short-term surgical procedures (Huff 1971).

Trichloroethylene has a molecular weight of 131.4. It is non-flammable and has a chloroform-like odor. Density at 20°C is 1.4649; boiling point at 760 mmHg is 86.7°; vapor density is 4.53 (air = 1.00) (Windholz 1976). At 25°C, 1 ppm in air is equivalent to 5.45 mg/m^3; the odor threshold is 0.5 mg/kg water (Van Gemert and Nettenbreijer 1977).

The solvent used in industry before the mid-1960s contained impurities, such as 1,1,2,2-tetrachloroethane, and some stabilizers, such as epichlorhydrin. A more pure product was obtained in the early 1960s due to a change in the manufacturing process (MRI 1979).

The U.S. produces approximately 234,000 metric tons of TCE a year (40 FR 48907, October, 1975). Volatilization of TCE during production and use is the major source of environmental levels of this compound. Trichloroethylene has been detected in air, water and marine organisms. Its detection in rivers, municipal water supplies, the sea and aquatic organisms indicates that TCE is widely distributed in the aquatic environment. The authors have concluded that it is not persistent in the environment and that there is no significant bioaccumulation in marine food chains (Pearson and McConnell 1975).

Recently, TCE has been detected in the groundwater of several states across the U.S. Region III of the USEPA reported high concentrations of TCE at several locations in Pennsylvania and Delaware. The concentration of TCE in these waters ranged from 18 to 22,000 ppb. How TCE entered the groundwater in these areas has not been determined.

III. PHARMACOKINETICS

A. Absorption

Several reports have indicated that TCE is absorbed into the bloodstream by all the three routes of entry, inhalation, oral and dermal. Information on the quantitative aspects of TCE absorption, however, is limited.

Soucek and Vlachova (1960) exposed three men and two women (average age of 21 years) to TCE vapors in an exposure chamber for five hours. The concentrations of TCE used in this experiment were 500, 850, 820 and 830 ug/L. The concentration of the TCE retained by the test subjects was calculated by subtracting the levels of TCE in the expired air from the concentrations in the exposure chamber. The method of analysis of TCE was not described. The authors calculated that the body retains an average of 65% of inhaled TCE. Soucek et al. (1952) recorded a range between 51% and 64%, with an average of 58%.

Data on absorption of ingested TCE are limited. Several reports concerning accidental ingestion of TCE that resulted in poisoning (Kleinfeld and Tabershaw 1954, Gibitz and Ploechal 1973) have provided evidence that TCE is absorbed via the G.I. tract.

The Dekant et al. (1984) excretion study provided a quantitative estimate of gastrointestinal absorption following administration of a single oral dose of TCE. A dose of 200 mg/kg of ^{14}C-TCE in corn oil was administered to two female Wistar rats (220 to 260 g) and three female NMRI mice (24 to 27 g). The amount of ^{14}C-radioactivity eliminated in expired air, urine and feces and that remaining in the carcass were measured 72 hours after dosing. Almost all (93% to 98%) of the administered radioactivity was recovered. A total of about 97% of the radioactivity was excreted by rats in exhaled air (53.9%), urine 41.2% and feces (1.8%). About 2.9% remained in the carcass. The mice excreted radioactivity in exhaled air (17.0%), urine (76.2%) and feces (4.9%). About 2% remained in the carcass. It can be inferred that about 98% of the administered dose in rats and 95% of that in mice was absorbed from the gastrointestinal tract within 72 hours.

Stewart and Dodd (1964) demonstrated that the alveolar breath concentration from skin exposure to TCE was only 0.5 ppm after subjects had immersed their thumbs for 30 minutes in a beaker containing the compound. Using alveolar breath levels to measure absorption and assuming no body retention, the authors stated that unless TCE was trapped against the skin, it was not absorbed in any significant quantities. Frant and Westendorp (1950) showed that when a volunteer's hands had been dipped into the solvent for 10 minutes, absorption through the skin was of minor importance; three days later, the trichloroacetic acid content in the urine was found to be only 1.5 mg/L. To ensure that the only mode of entry of TCE was through the skin, the subject wore a protective gas mask during the experiment. Schwander (1936) demonstrated that TCE penetrated the skin of rabbits and was detected in the expired air.

B. Distribution

After absorption, TCE enters the blood and is distributed to the various tissues and organs. Most of the data on tissue levels have been obtained through inhalation studies. There are limited data on disposition of ingested TCE, although there is substantial evidence that TCE enters the bloodstream after ingestion.

Zenick et al. (1984) determined tissue levels of TCE and the major metabolites, trichloroethanol (TCOH) and trichloroacetic acid (TCA), in Long-Evans rats (three males/dose group, 100 days of age). Doses of 0, 10, 100 or 1,000 mg TCE/kg/day were administered by gavage for five days/week for six weeks. Marginal increases in TCE levels were detected in the 10 mg/kg/day and 100 mg/kg/day dose groups compared to controls (see Table III-1). A marked increase in TCE levels of most tissues was observed in the highest dose group. Trichloroethylene was distributed to all tissues examined with the highest concentrations in the fat, kidney, lung, adrenals, vas deferens,

Table III-1 Median Blood and Tissue Levels of TCE and Metabolites in Male Rats

Organ	Treatment Groups[a]											
	Control (corn oil)			10 mg/kg/day			100 mg/kg/day			1,000 mg/kg/day		
	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)
Blood[b]	1.21	0.17	ND[c]	1.39	2.5	0.13	1.20	14.32	1.66	17.90	28.13	8.00
Heart	1.04	0.007	ND	2.67	5.5	0.03	2.76	5.42	1.61	7.70	7.92	4.46
Testis	1.89	0.06	ND	2.16	1.06	0.20	3.42	10.56	1.90	9.40	13.57	7.57
Vas deferens	8.2	0.22	0.05	8.36	2.47	0.02	5.36	8.24	5.35	13.65	21.09	6.25
Seminal vesicle	2.40	0.023	0.04	2.95	0.89	0.23	3.86	8.97	4.28	8.05	21.86	28.80
Prostrate	1.69	0.04	0.03	3.09	0.96	0.39	4.32	6.49	5.89	8.85	15.79	17.58
Epididymis	3.17	0.06	0.02	7.77	0.58	0.03	6.78	7.90	5.45	9.17	9.84	5.49
Adrenals	8.45	ND	ND	6.63	1.81	1.72	8.62	5.94	0.86	6.52	9.06	1.82
Fat	53.95	0.06	0.06	55.24	0.110	0.24	52.76	3.78	3.76	265.90	4.19	11.04
Liver	1.70	0.001	0.012	3.10	1.16	1.81	4.67	13.82	11.76	13.64	9.44	41.57
Kidney	0.145	0.01	0.04	5.0	1.96	2.56	4.12	10.28	14.83	18.47	28.25	81.17
Muscle	0.83	0.01	0.01	1.94	0.86	0.02	3.96	9.21	4.06	3.57	6.57	4.80
Lung	2.48	0.07	ND	3.99	2.71	0.13	2.65	6.82	1.68	15.48	10.65	4.99
Brain	1.12	0.006	ND	1.91	0.53	ND	2.33	5.20	3.11	10.42	3.37	3.97

[a] Daily oral dosing was five days/week for six weeks. There were three male rats per dose level.
[b] Values given for blood are ug/mg.
[c] ND = Not detected

Adapted from Zenick et al. (1984).

epididymis, brain and liver. Dose related trends in tissue distribution were evident for TCA and TCOH. The TCA and TCOH tissue levels were several order of magnitudes greater than those in controls.

Manson et al. (1984) analyzed TCE, TCOH and TCA levels in tissues of non-pregnant rats administered 0, 10, 100 or 1,000 mg TCE/kg/day for five days/week for three weeks. Groups of three female Long-Evans rats were administered the TCE doses by gavage with a corn oil vehicle. Tissue levels were analyzed by gas chromatography.

Several tissues in control animals contained measurable levels of TCE and metabolites. The authors proposed several explanations for the contamination. Possible exposures to TCE in food, water or the corn oil vehicle were suggested to account for tissue levels in controls. The vehicle was contaminated with relatively high TCE concentrations (13.5 ug/mL). Another explanation was that controls housed in the same room as treated animals could have been exposed to TCE via expired air from treated animals.

The tissue levels of TCE and metabolites in controls and treatment groups are listed on Table III-2. Fat, adrenals and ovaries tended to contain high TCE levels among all dose groups. The TCE levels in tissues of the 10 mg/kg/day group were comparable to those of controls while levels of metabolites

Table III-2 Mean Blood and Tissue Levels of TCE and Metabolites in Female Rats

Organ	Treatment Groups[a]											
	Control (corn oil)			10 mg/kg/day			100 mg/kg/day			1,000 mg/kg/day		
	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)	TCE (ug/g)	TCA (ug/g)	TCOH (ug/g)
Heart	0.82	0.19	ND[b]	0.75	1.29	0.40	1.12	4.78	3.04	47.95	6.63	11.30
Brain	1.15	0.61[c]	ND	0.56	0.45	0.41	0.76	1.59	1.58	56.03	8.26	8.19
Lung	1.42	1.95[c]	0.05	0.50	0.97	0.73	2.79	10.73	3.52	32.84	22.56	13.91
Liver	1.42	ND	ND	0.74	2.62	1.81	1.80	10.21	10.27	98.09	17.10	47.15
Muscle	1.35	ND	ND	0.74	1.04	0.28	2.32	4.84	2.12	22.88	11.19	9.42
Kidney	1.85	0.34[c]	ND	0.83	3.05	2.97	3.97	13.76	18.90	55.68	34.74	63.92
Adrenals	3.53	0.10[c]	0.12[c]	1.01	1.31	0.44	5.84	6.59	2.43	63.98	10.26	8.55
Fat	1.01	0.03	0.05[c]	3.04	1.06	1.11	36.47	4.07	4.43	1478.11	2.98	14.64
Ovaries	1.69[c]	ND	ND	1.81	1.49	0.48	4.73	7.67	2.29	13.68	16.67	8.35
Uterus	1.68	0.41[c]	ND	0.97	2.72	0.46	2.87	12.48	2.35	2.68	21.79	12.58
Blood	0.36	0.10	0.01	0.48	4.80	0.59	0.94	15.41	2.57	45.40	28.92	11.80

[a] Daily oral dosing was five days/week for three weeks. There were three female rats per dose level.

[b] ND = Not detected.

[c] Detectable amounts found only in a single sample.

Adapted from Manson et al. (1984).

were elevated. The parent compound and metabolites were elevated in all tissues in the 100 mg/kg/day group. All tissues except the brain had higher TCE levels than that of blood in this dose group. In the 1,000 mg/kg/day dose group, the TCE and metabolite levels in tissues were increased but the organ-to-blood ratios were constant or decreased relative to ratios in the 100 mg/kg/day group (see Table III-3). The authors concluded that the rate of uptake of TCE and metabolites did not increase when the dose of TCE increased.

Kulkarni (1944) determined TCE blood and tissue levels of dogs, rabbits, guinea pigs and cats after exposure to TCE vapors. The lethal TCE blood concentration in dogs was found to be 100 to 110 mg/100 mL blood; for chloroform anesthesia, it was 60 to 65 mg/100 mL blood. At the anesthetic stage, TCE blood levels were 24 to 37, 23 to 28, 14 to 18 and 25 to 32 mg/100 mL blood for dogs, rabbits, guinea pigs and cats, respectively. The blood-brain ratio at anesthetic dosages was approximately 1:2 for both guinea pigs and dogs. Guinea pigs and rats were used by Fabre and Truhaut (1952) to determine how TCE vapors distributed to the tissues. Guinea pigs were exposed to 600 to 900 mg/m^3 for 5 to 23 days (4.5 to 5.25 hours/day). Biological effects, per se, were not evaluated in this study; rather, tissue distribution was assessed. A trend for distribution of TCE can be observed in this study. Trichloroethylene was present in most of the examined tissues; the greatest concentrations were in fat, followed by adrenals,

Table III-3 Organ to Blood Ratios of TCE and Metabolites in Female Tissues[a]

Tissues	10 mg/kg			100 mg/kg			1,000 mg/kg		
	TCE	TCA	TCOH	TCE	TCA	TCOH	TCE	TCA	TCOH
Heart	1.7	0.3	0.7	1.1	0.3	1.2	1.1	0.2	1.0
Brain	1.3	0.1	0.3	0.9	0.1	0.6	1.2	0.3	0.7
Lung	1.0	0.2	1.3	2.9	0.7	1.4	0.8	0.8	1.2
Liver	1.7	0.5	3.0	1.9	0.7	4.0	2.3	0.6	4.1
Muscle	1.7	0.2	0.5	2.3	0.3	0.8	0.5	0.4	0.8
Kidney	1.9	0.4	5.0	3.9	0.9	7.3	1.3	1.2	5.4
Adrenals	1.7	0.3	0.7	6.0	0.4	0.9	1.3	0.3	0.7
Fat	7.0	0.2	1.9	38.8	0.3	1.7	37.3	0.1	1.3
Ovaries	4.8	0.3	0.8	5.1	0.5	0.8	0.4	0.6	0.7
Uterus	2.2	0.6	0.7	2.9	0.8	0.9	0.1	0.8	1.1
Mean	2.5	0.3	1.5	6.6	0.5	2.0	4.6	0.5	1.7

[a] Results are presented as the mean ratio values in each organ relative to the blood.

Adapted from Manson et al. (1984).

ovaries, kidneys, lungs, brain and liver. A metabolite, trichloroacetic acid, was found in the greatest concentrations in the adrenals, ovaries, spleen, kidneys, lungs, adipose tissue and brain. After acute exposure to TCE, the greatest amount of trichloroacetic acid was present in the spleen. After repeated exposure, the largest amount of acid was present in the lungs.

To study the effect of embalming on TCE tissue concentration, Stewart et al. (1964) administered 1 and 2 mL of TCE orally to dogs weighing 8 and 10.2 kg, respectively. The animals were sacrificed 16 hours after exposure, and the tissue levels were determined 4, 10 and 21 days later, utilizing gas chromatographic techniques. Omental fat contained the highest level of TCE.

Tissue distribution of TCE in humans has been studied by several investigators (Powell 1945, Astrand and Ovrum 1976, Versterberg and Astrand 1976, Clayton and Parkhouse 1962, Laham 1970, Beppu 1968). The data were collected both from patients under anesthesia and from autopsies of human subjects. As with animals, inhaled TCE vapors were demonstrated to be readily absorbed into the bloodstream of humans.

In an inhalation study by Powell (1945), 12 patients were exposed (during anesthesia) to 1.5 to 2.5% TCE for at least half an hour. Concentrations in venous blood varied between

6.5 and 12.5 mg/100 mL. The blood concentration was reduced to 1 mg % within 3 hours and to 0.1% within 24 hours. Lower TCE blood concentrations (2.8 ± 1.14 mg/100 mL) were reported, however, in women after TCE anesthesia administered during vaginal deliveries. These women inhaled TCE vapors for an average of 34.7 minutes (Beppu 1968). When the inhalation time for TCE anesthesia was reduced to 10 to 19 minutes, maternal venous blood ranged from 0.67 to 8 mg TCE/100 mL blood (Laham 1970). Clayton and Parkhouse (1962) recorded 2.2 to 11.3 mg TCE/100 mL in venous blood of subjects who inhaled 0.5 to 1.0 TCE concentration volume/volume percent for 20 to 25 minutes.

Trichloroethylene is readily transported from mother to fetus. Beppu (1968) noted that TCE may be transported across placental barriers in pregnant women. The mean inhalation time of 34 subjects was 34.7 minutes; the mean concentrations of TCE were 2.80 ± 1.14 mg/100 mL in the femoral (cubital) maternal arteries, 2.36 ± 1.17 mg/100 mL in the cubital maternal veins, 1.83 ± 1.08 mg/100 mL in the umbilical veins and 1.91 ± 0.95 mg/100 mL in the umbilical arteries. The concentration of TCE in fetal blood was lower than that of the mother's blood. Laham (1970) obtained similar results from studies on placental transfer of TCE. Ten case studies involving women from 20 to 28 years old were reported. The intermittent inhalation technique was used to produce anesthesia; duration of inhalation was between 10 and 19 minutes. Maternal venous blood contained 0.67 to 8 mg TCE/100 mL of blood, whereas fetal blood concentrations ranged from 1 to 5.20 mg TCE/100 mL.

TCE has been detected in human tissues. Specimens from eight humans were examined post-mortem by McConnell et al. (1975). The body fat, liver, kidney and brain tissue samples contained TCE, indicating uptake by these tissues (Table III-4).

C. Metabolism

Studies indicate that TCE is metabolized to trichloroethylene oxide (2,2,3-trichlorooxirane), trichloroacetaldehyde, trichloroacetic acid, monochloroacetic acid, trichloroethanol and trichloroethanol glucuronide. These metabolites have been obtained in both in vivo and in vitro experiments, utilizing both experimental animals and human systems. In general, the metabolites reported in the animal systems were qualitatively similar to those found in humans.

Dekant et al. (1984) demonstrated that TCE is primarily metabolized to trichloroethanol, trichloroacetic acid and N-(hydroxyacetyl)-aminoethanol (HAAE) in mice and rats following administration of a single oral dose. Two female Wistar rats and three female NMRI mice were administered 200 mg/kg of ^{14}C-TCE in corn oil by gavage. About 52% and 11% of the ^{14}C-TCE was not metabolized in rats and mice, respectively, and was excreted in expired air. About 2% and 6%, respectively,

Table III-4 Occurrence of Trichloroethylene in Human Tissue[a]

Age of Subject	Sex	Tissue	TCE (ug/kg)
76	F	Body fat	32
		Kidney	< 1
		Liver	5
		Brain	1
76	F	Body fat	2
		Kidney	3
		Liver	2
		Brain	< 1
82	F	Body fat	1.4
		Liver	3.2
48	M	Body fat	6.4
		Liver	3.5
65	M	Body fat	3.4
		Liver	3.5
75	M	Body fat	14.1
		Liver	5.8
66	M	Body fat	4.6
74	F	Body fat	4.9

[a] Post-mortem samples taken from subjects of unreported work history or TCE exposure who lived in north-western England; isolation accomplished by solvent extraction and column chromatography; samples analyzed by gas-liquid chromatography using an electron capture detector with confirmation by mass spectroscopy.

Adapted from McConnell et al. (1975).

was metabolized to $^{14}CO_2$ and excreted in expired air. The majority of the remainder of the TCE was metabolized and excreted in the urine. Urinary TCE metabolites accounted for 41% and 76% of the recovered ^{14}C-activity in rats and mice, respectively. The urinary metabolites and relative portions of ^{14}C-activity are provided in Table III-5.

A proposed pathway for the metabolism of TCE is given in Figure III-1. According to this pathway, the first step in the biotransformation of TCE is the formation of 2,2,3-trichlorooxirane by the epoxidation of the double bond. Uehleke and Poplawski-Tabarelli (1977) compared the absorption spectra at 451 to 452 nm of the incubated rabbit liver microsomes with TCE alone and with 2,2,3-trichlorooxirane added to reduce the suspension of rabbit liver microsomes. Both preparations had identical spectra. Trichloroethylene did not form a ligand absorption spectrum with hepatic microsomes reduced by dithionite or in anaerobic incubates in the presence of NADPH. _In vivo_ experiments have not isolated or characterized 2,2,3-trichlorooxirane, probably due to the unstable nature of the compound.

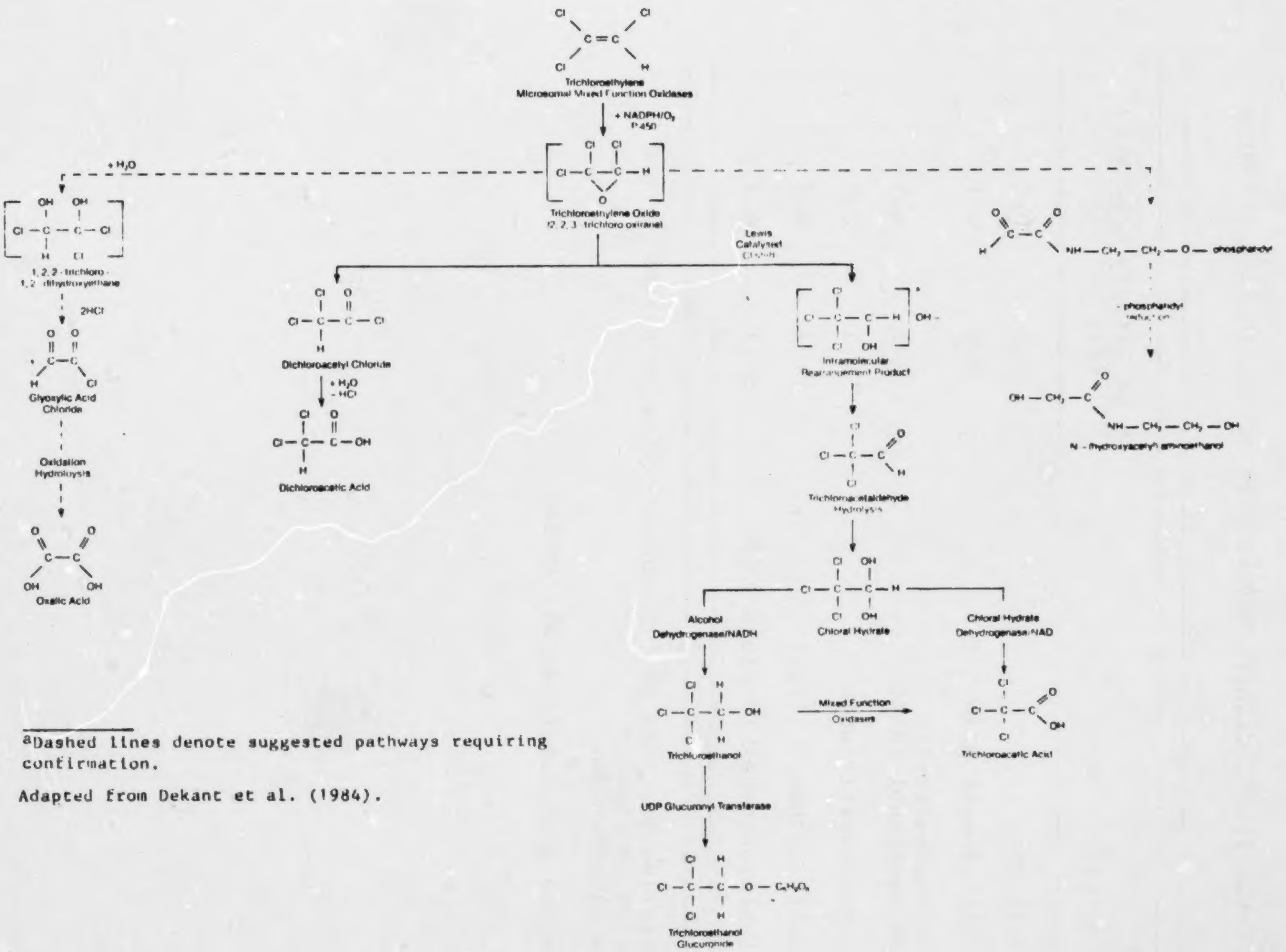
Henschler et al. (1979) studied the reactivity of synthesized 2,2,3-trichlorooxirane in an _in vitro_ aqueous system to simulate physiological conditions that this compound may encounter as it forms _in vivo_. The 2,2,3-trichlorooxirane decomposed rapidly by means of C-C and C-Cl fissions to

Table III-5 Urinary Metabolites of TCE in Rats and Mice

Metabolite	% of urinary acitivity[a] rats	mice
Oxalic acid	1.3	0.7
Dichloroacetic acid	2.0	0.1
N-(Hydroxyacetyl)-aminoethanol (HAAE)	7.2	4.1
Trichloroacetic acid	15.3	0.1
Trichloroethanol (free)	11.7	0.1
Trichloroethanol (conjugated)[b]	61.9	94.2

[a] Relative portions of ^{14}C-radioactivity in pooled 72 hour urine samples.
[b] As glucuronide.

Adapted from Dekant et al. (1984).


Trichloroethylene
Microsomal Mixed Function Oxidases
+ NADPH/O2
P-450
Trichloroethylene Oxide
(2, 2, 3 - trichloro oxirane)
+ H2O
1, 2, 2 - trichloro - 1, 2 - dihydroxyethane
2HCl
Glyoxylic Acid Chloride
Oxidation
Hydrolysis
Oxalic Acid
Dichloroacetyl Chloride
+ H2O
- HCl
Dichloroacetic Acid
Lewis Catalyst
Intramolecular Rearrangement Product
Trichloroacetaldehyde
Hydrolysis
Chloral Hydrate
Alcohol Dehydrogenase/NADH
Chloral Hydrate Dehydrogenase/NAD
Trichloroethanol
Mixed Function Oxidases
Trichloroacetic Acid
UDP Glucuronyl Transferase
Trichloroethanol Glucuronide
- phosphatidyl reduction
N - (hydroxyacetyl) aminoethanol

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[a] Dashed lines denote suggested pathways requiring confirmation.

Adapted from Dekant et al. (1984).

Figure III-1 Proposed Intermediary Metabolism of Trichloroethylene[a]

predominantly single-carbon compounds (i.e. carbon monoxide and formate) and hydrochloric acid. These *in vitro* observations, however, did not support epoxidation as the metabolic pathway of TCE since metabolic transformations *in vivo* are exclusively to trichlorinated two-carbon compounds without C-Cl fission. In addition, carbon monoxide does not form during microsomal tri-oxidation. The authors suggested, however, that the 2,2,3-trichlorooxirane rearranges, contrary to expectation due to thermal rearrangement behavior, to chloral in presence of Lewis Acids. The apparent discrepancy was explained as follows: TCE is epoxidised by mixed function oxygenases; the catalytic action of trivalent iron of P-450 facilitates the immediate rearrangement of 2,2,3-trichlorooxirane to the chloral within the hydrophobic portion of the enzyme, so that decomposition reactions described above do not occur.

Dekant et al. (1984) suggested that rearrangement of the epoxide outside the hydrophobic premise of cytochrome P-450 without interaction of metals yields dichloroacetyl chloride. Dichloroacetyl chloride reacts with water to form dichloroacetic acid.

Intramolecular rearrangement of trichloroethylene oxide in the presence of metals and hydrolysis may result in the formation of chloral hydrate. Experiments conducted by Daniel (1963) suggest that the rearrangement favors the pathway leading to the formation of chloral hydrate and the subsequent metabolites, trichloroethanol and trichloroacetic acid. The author showed that chlorine attached to TCE is not removed during biotransformation in rats exposed to ^{36}Cl-labeled compound. Approximately 93% of the ^{36}Cl-labeled TCE administered by stomach tube was excreted unchanged through the lungs or in the urine as trichloroethanol and trichloroacetic acid. The specific activities of metabolic trichloroacetic acid and trichloroethanol were shown to be the same as that of the administered TCE, thus demonstrating an intramolecular rearrangement of chloride.

Chloral hydrate has been suggested as an intermediate in the metabolic pathway of TCE since 1949. Later, Leibman (1965) and Byington and Leibman (1965) demonstrated the transformation of the TCE to chloral hydrate. These workers utilized liver microsomes of rats, rabbits and dogs in a reaction requiring NADPH and oxygen. Ikeda and Imamura (1973) confirmed this finding using rat liver microsomes. Chloral hydrate was identified _in vivo_ by Kimmerle and Eban (1973a) using rats exposed to TCE vapors. Chloral hydrate as a metabolite of TCE in the plasma of human subjects following TCE anesthesia was demonstrated by Cole et al. (1975).

The next step of the metabolic process in Figure III-1 involves biotransformation of chloral hydrate to trichloroethanol (by a reduction reaction) and trichloroacetic acid (by oxidation processes). Trichloroacetic acid was identified by the Fujiwara test in the urine of dogs exposed to TCE vapors (Barret and Johnston 1939). The identity of trichloroacetic acid was confirmed by its melting point and mixed melting point with an authentic sample of trichloroacetic acid (Powell 1945). The quantitative relationship of formation and the course of elimination in the urine of the metabolites, including trichloroacetic acid, were determined by Soucek and Vlachova (1959, 1960). Three men and two women, with an average age of 21 years, were exposed to TCE vapors. Their urine was analyzed for monochloroacetic acid, trichloroacetic acid and trichloroethanol. Sex-related differences in the metabolism of TCE were not noted.

After oral administration of TCE to rabbits, Ogata and Saeki (1974) reported the presence of monochloroacetic acid and chloral hydrate in the blood serum. Because of its short half-life, however, chloral hydrate does not remain in the body for a long length of time.

Dekant et al. (1984) proposed metabolic pathways to account for other major TCE metabolites detected in urine of rats and mice given a single oral dose of TCE (see Figure III-1). The epoxide may undergo enzymatic or non-enzymatic cleavage and produce 1,2,2-trichloro-1,2-dihydroxyethane, an unstable vicinal diol. This diol may spontaneously eliminate two hydrogen chloride molecules and form glyoxylic acid chloride. This compound reacts with water and is oxidized to form oxalic acid. The mechanism for formation of HAAE, another major metabolite, has not been established.

Miller and Guengerich (1982, 1983) provided evidence that TCE-oxide is not an obligate intermediate in the formation of chloral and proposed an alternative metabolic scheme (see Figure III-2). Miller and Guengerich (1982) observed that TCE was metabolized by cytochrome P-450 containing mixed-function oxidase systems to chloral, glyoxylic acid, formic acid, CO and TCE oxide. The _in vitro_ study of TCE-oxide degradation revealed that glyoxylic acid and dichloroacetic acid formed under acidic conditions and formic acid and carbon monoxide formed under neutral and basic conditions. The TCE-oxide did not form chloral in aqueous systems even when iron salts or purified cytochrome P-450 was present. The epoxide rearranged to chloral in some non-aqueous systems and trivalent iron salts catalyzed this rearrangement to the greatest extent. Studies of the kinetics for oxidation of TCE to TCE-oxide by cytochrome P-450 and degradation of the epoxide were the basis of Miller and Guengerich (1982) conclusion that the epoxide is not the obligate intermediate and that chlorine migration occurs in an oxygenated TCE-cytochrome P-450 intermediate to form chloral. Miller and Guengerich (1983) conducted additional studies in several _in vitro_ systems involving liver cytochrome P-450, liver microsomes, lung microsomes and isolated hepatocytes. The studies indicated that TCE metabolism involves formation of a complex with oxygenated cytochrome P-450 which, by

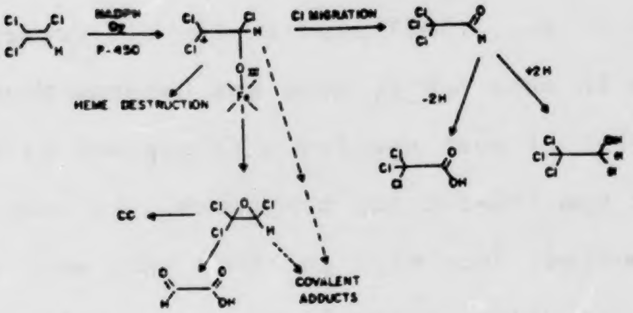
NADPH
O2
P-450
Cl MIGRATION
HEME DESTRUCTION
-2H
+2H
CC
COVALENT ADDUCTS

Figure III-2 Postulated Pathways for TCE Metabolism

Adapted from Miller and Guengerich (1983).

rearrangement, may result in: suicidal heme destruction; chloral formation, which is reduced to trichloroethanol and conjugated to form glucuronide or oxidized to trichloroacetic acid; formation of TCE oxide, which decomposes to carbon monoxide and glyoxylate; and metabolites that bind irreversibility to DNA, RNA and protein (see Figure III-2).

Stott et al. (1982) studied the differences in TCE metabolism in male $B_6C_3F_1$ mice and Osborne-Mendel rats. Groups of 16 animals of each species were exposed by inhalation to 10 ppm or 600 ppm ^{14}C-TCE for six hours. At completion of the exposure period, four mice and four rats were selected and placed in individual metabolism cages. Elimination of ^{14}C-activity in urine, feces and expired air was measured for 50 hours post-exposure. The animals were sacrificed and residual activity in the carcass was measured. In mice exposed to 10 or 600 ppm TCE, about 98% to 99% of the total body burden was metabolized within 50 hours (see Table III-6). About 9% to 10% of ^{14}C-TCE was metabolized to $^{14}CO_2$ and excreted in expired air. About 1% to 2% was not metabolized and was excreted as unchanged ^{14}C-TCE in expired air. The authors concluded that the metabolism of the $B_6C_3F_1$ mouse did not appear to be saturated at the 600 ppm TCE exposure level since the routes of excretion and total metabolism of ^{14}C-TCE in the 10 ppm and 600 ppm groups were similar.

Table III-6 Recovery of Radioactivity from Mice and Rats Exposed to ^{14}C-TCE by Inhalation[a]

	Mice		Rats	
	(10 ppm)	(600 ppm)	(10 ppm)	(600 ppm)
	% Recovered[b]	% Recovered	% Recovered	% Recovered
Expired 1,1,2-TCE	0.79	2.4	2.1	21.1
CO_2	9.4	9.5	4.8	2.9
Urine	74.0	72.6	63.1	55.3
Feces	3.7	3.7	7.0	3.6
Cage wash[c]	1.4	2.7	1.0	1.4
Skin	3.0	1.8	10.6	7.1
Liver	3.1	2.3	2.8	1.6
Kidney	0.39	0.35	0.37	0.16
Carcass	1.9	4.6	7.9	7.0
Total metabolized	99.2	97.6	97.8	78.9

[a] Average of four animals exposed to 10 or 600 ppm ^{14}C-TCE for six hours.

[b] Percentage of recovered radioactivity at 50 hours post-exposure.

[c] Primarily due to urine.

Adapted from Stott et al. (1982).

In contrast, the Osborne-Mendel rat demonstrated characteristics of saturation at the 600 ppm exposure level. The total metabolism of ^{14}C-TCE in the 600 ppm group (79%) was decreased relative to that of the 10 ppm group (98%). The elimination of unchanged ^{14}C-TCE by the 600 ppm group (21%) was increased by 10-fold over that in the 10 ppm group (2.1%).

Comparison of TCE metabolism in mice and rats on the basis of ^{14}C-TCE/kg revealed that mice metabolized 123% ($P < 0.01$) more TCE than rats exposed at the 10 ppm level and 262% ($P < 0.01$) more than rats at the 600 ppm exposure level. Thus, the mice metabolized inhaled TCE to a greater extent than rats.

Stott et al. (1984) observed that mice also metabolized more inhaled TCE to a hepatic macromolecular binding metabolite than rats. The 12 animals of each species not selected for the metabolism study (described above) were examined for macromolecular binding. A group of four mice and four rats was sacrificed at 0, 6 and 24 hours post-exposure. Tissue analysis of kidneys and livers determined irreversible binding of radioactivity. The mice metabolized more ^{14}C-TCE to a reactive macromolecular binding metabolite than rats at both low and high exposure levels. The species differences in binding in both the kidney and liver were most apparent at the high dose (600 ppm level); mice had significantly ($P < 0.01$) more binding (about 3 to 4 times) in liver and kidney tissue than that in rats. This was presumably due to the saturation of the TCE metabolic activation pathway in rats.

D. Elimination

Trichloroethylene and its metabolites are excreted in urine, by exhalation and, to a lesser degree, in sweat, feces and saliva. Trichloroethanol, trichloroethanol glucuronide, monochloroacetic acid and trichloroacetic acid appear in the urine immediately after exposure begins. Monochloroacetic acid is excreted from the organism the fastest, followed by trichloroethanol, trichloroethanol glucuronide and trichloroacetic acid. On the other hand, TCE is excreted in the urine in small amounts (Soucek and Vlachova 1959).

Urinary elimination of TCE metabolites has been investigated in experimental animals by several researchers (Friberg et al. 1953, Forssmann and Holmquist 1953, Kimmerle and Eben 1973a, Ogata and Saeki 1974). Rats exposed to TCE vapors excreted Fuhiwara-positive reaction products which were calculated to be trichloroacetic acid (Friberg et al. 1953, Forssmann and Holmquist 1953). Kimmerle and Eben (1973a) detected trichloroacetic acid and trichloroethanol glucuronide in the urine of rats given TCE by inhalation. Trichloroacetic acid was determined colorimetrically, whereas trichloroethanol glucuronide was analyzed by gas chromatography after enzymatic hydrolysis of the urine samples. After oral administration of TCE to rabbits, the following metabolites, in order of decreasing concentration, were detected in the urine: chloral hydrate > trichloroethanol, monochloroethanol and monochloroacetate > trichloroacetate (Ogata and Saeki 1974).

Dekant et al. (1984) studied excretion of radiolabeled TCE and metabolites in female mice and rats. A single oral ^{14}C-TCE dose of 200 mg/kg/bw in corn oil was administered by gavage to two female Wistar rats (220 to 260 g) and to three female NMRI mice (24 to 27 g). The amount of ^{14}C-radioactivity eliminated by various routes 72 hours after dosing was measured (see Table III-7). Almost all (93% to 98%) of the administered radioactivity was recovered. The rats exhaled 52% of the radioactivity as unchanged ^{14}C-TCE and excreted 41.2% as TCE metabolites in urine. The mice exhaled 11% as unchanged ^{14}C-TCE and excreted 76.2% as TCE metabolates in urine.

Human volunteers and/or patients were used to study the elimination of TCE after inhalation exposure. Depending on the concentration and the exposure time, significant quantities of TCE were eliminated by the lungs, following the general rule that low molecular weight compounds are preferentially excreted from the lungs. The amount of TCE excreted through the lungs ranged from 40% to 70% (Bartonicek 1962, Ogata et al. 1971, Soucek and Vlachova 1960).

Table III-7 Elimination of ^{14}C-Radioactivity After ^{14}C-TCE Administration to Rats and Mice

Route	Time (Hours)	Recovered Radioactivity (%)[a] Rats	Recovered Radioactivity (%)[a] Mice
Exhaled Air			
^{14}C-TCE	0 - 72	52.0	11.0
$^{14}CO_2$	0 - 72	1.9	6.0
Urine	0 - 12	22.0	30.0
	12 - 24	17.0	39.0
	24 - 48	2.0	6.0
	48 - 72	0.2	1.0
Feces	0 - 72	1.8	4.9
Total Excreted	0 - 72	96.9	97.9
Retained in Carcass	-	2.9	2.0
Residual in Cage	-	0.2	0.1

[a] About 93% to 98% of administered radioactivity was recovered.

Adapted from Dekant et al. (1984).

Soucek and Vlachova (1960) examined the excretion time and percent excretion of monochloroacetic acid, trichloroacetic acid and trichloroethanol in humans (three men and women) exposed for five hours to dosages ranging from 440 to 850 mg/m^3 TCE. The excretion of the metabolites was measured over the next 7 to 14 days. The quantities of the metabolites excreted were not related to individual dosages. Monochloroacetic acid was shown to be excreted in the first few minutes after exposure. Excretion of monochloroacetic acid was maximal at the end of the exposure and continued for 48 to 168 hours, with an average of 4.8 days (112 hours). Monochloroacetic acid constituted about 4% of the retained TCE. Trichloroacetic acid appeared in the urine immediately after inhalation, and its concentration slowly rose due to its ability to accumulate in the body. Maximal excretion occurred within 24 to 48 hours and lasted for 520 hours. The fall in the rate of excretion was considered to be the sum of two exponential rates (phases). The first phase lasted about five days, and the second phase lasted approximately 14 days. Trichloroacetic acid constituted 10% to 30% (19% average) of the retained vapor. Trichloroethanol was also excreted within the first few minutes of exposure. Excretion of Trichloroethanol rose very rapidly and reached its maximum a few hours after exposure. The excretion time was 312 to 390 hours (average of 350 hours). A decrease in the excretion rate appared to be the sum of two exponential rates. The first phase lasted three to four days, while the second phase lasted seven to nine days. The total quantity of trichloroethanol excreted was between 32% and 59% of the TCE retained; the average was 50%. The total quantity of these three metabolites excreted in the urine of humans amounted to from 43% to 100% of the absorbed TCE. The ratio of these three metabolites was found to be monochloroacetic acid:trichloroacetic acid:trichloroethanol = 1:5:12.

Bartonicek (1962) and Ogata et al. (1971) confirmed Soucek and Vlachova's findings. Eight volunteers (both males and females) were exposed to 1,042 mg/m^3 TCE for five hours by Bartonicek (1962). Of the retained TCE, 38.0% to 49.7% and 27.4% to 35.7% were excreted in urine as trichloroethanol and trichloroacetic acid, respectively. The amount of TCE eliminated via the lungs was not determined. In the same experiment, Bartonicek found that trichloroethanol and trichloroacetic acid were excreted in the feces, for a total of 8.4%. The length of time and the time intervals at which expired air was analyzed for TCE were not provided. Therefore, the amount of TCE absorbed cannot be determined accurately; there is a possibility of reaching a steady state between the blood concentration and the inhaled TCE concentration.

Ogata et al. (1971) conducted two separate experiments on 13 male subjects exposed to approximately 474 and 927 mg/m^3 TCE. One group of five people (A) remained in the exposure chamber for three hours in the morning and four hours in the afternoon at an exposure of 927 mg/m^3. A second group of four people (B) were exposed to 474 mg/m^3, but they remained in the chamber for only three hours (in the morning). Urine was collected for 100 hours after the initial exposure. In Groups A and B, the concentrations of trichloroethanol were maximum one to three hours after exposure, and trichloroacetic acid concentrations were maximum 42 to 69 hours after exposure. The excretion rates of trichloroacetic acid and trichloroethanol returned to normal after 92 hours. The total amounts of trichloroethanol and trichloracetic acid recovered in the urine were 44% and 18.1%, respectively, for the seven-hour exposure. The final amounts of trichloroethanol and trichloroacetic acid recovered in the three-hour exposure to 927 mg/m^3 were 53% and 21.9%, respectively.

The levels of TCE metabolites in the urine of humans have been recorded by many researchers. Ikeda and Ohtsuji (1972) conducted two separate experiments on male workers exposed to TCE vapors (1,090 mg/m^3) for eight hours and recorded the excretion of the metabolites in the urine. In the first experiment, six workers were exposed intermittently to 54.5 to 272.5 mg/m^3 of the solvent. Total trichloro-compounds varied from 38 to 376 mg/L, trichloroethanol varied from 11 to 281 mg/L, and trichloroacetic acid varied from 18 to 95 mg/L in the urine. In the second experiment, 14 workers were exposed intermittently to a range of from 650 to 1,363 mg/m^3 TCE. The urinary metabolites ranged from 55 to 487 mg/L for total trichloro-compounds, from 33 to 347 mg/L for trichloroethanol and from 22 to 177 mg/L for trichloroacetic acid. The overall time during which these urinary metabolites were measured was not given.

Surveys were conducted by Ikeda et al. (1972) on 85 male industrial workers (36 controls) under working environments. The urinary excretion of metabolites was recorded as total trichloro-compounds. The results are summarized in Table III-8 and show that metabolite concentration increased as exposure concentration increased.

Sukhanova and Burdygina (1971) measured the metabolite level in the urine of students during their four months of apprenticeship in a plant which used TCE. The content of metabolites in the urine increased significantly. After four months, the level of metabolites found in the urine of students ranged from 2.3 to 65.6 mg/L.

Five male volunteers were subjected to 1,090 mg/m^3 TCE for seven hours/day for five days (Stewart 1968). Before, after and during exposure, 24-hour urine samples were collected and analyzed for trichloroacetic acid and trichloroethanol. The results are summarized in Table III-9.

A study conducted by Friberg et al. (1953) showed similar results. Three people were exposed to TCE concentrations ranging from 100 to 150 ppm for seven hours daily for one week.

Table III-8 Average Metabolite Concentrations in Urine of Workers Exposed to Various Concentrations of Trichloroethylene

Number of people exposed	concentration (ppm)[a]	Time exposed	Metabolite concentrations (mg/L)		
			Total Trichloro-compounds	Trichloroethanol	Trichloroaceti acid
36	0	8 hr/day, 6 days/wk	1	0	1
9	3	8 hr/day, 6 days/wk	39.4	25.1	12.7
5	5	8 hr/day, 6 days/wk	45.6	24.9	20.2
6	10	8 hr/day, 6 days/wk	60.5	42.0	17.6
4	25	8 hr/day, 6 days/wk	164.3	77.3	77.2
4	40	8 hr/day, 6 days/wk	324.9	220.3	90.6
5	45	8 hr/day, 6 days/wk	399.0	256.7	138.4
5	50	8 hr/day, 6 days/wk	418.9	267.3	146.6
5	60	8 hr/day, 6 days/wk	468.0	307.9	155.4
4	120	8 hr/day, 6 days/wk	915.3	681.8	230.1
4	175	8 hr/day, 6 days/wk	1,210.9	973.1	235.8

[a] The parts per million of solvent in the air was measured using Kitagawa (1961) detection tubes. At least five determinations were made, and the averages were recorded.

From Ikeda et al. (1972).

Table III-9 Urinary Excretion of Trichloroacetic Acid and Trichloroethanol in Five Subjects During and Following Trichloroethylene Exposure[a]

Time	Metabolite concentration (mg/L) Trichloroacetic acid	Metabolite concentration (mg/L) Trichloroethanol
1st Exposure day	51 (34- 84)	308 (179-480)
2nd Exposure day	175 (113-238)	359 (294-480)
3rd Exposure day	229 (148-416)	399 (294-546)
4th Exposure day	306 (249-439)	538 (294-822)
5th Day following last exposure	50 (35- 61)	15 (10- 18)
12th Day following last exposure	8 (2- 22)	14 (1- 37)

[a] Subjects were exposed to 200 ppm TCE for seven hours/day for five days.

From Stewart (1968).

During the later days of the study, 250 to 500 mg of trichloroacetic acid per liter of urine was excreted. Frant and Westendorp (1950) calculated that if people were exposed to 100 ppm of TCE for several days, they would excrete about 200 mg/L of trichloroacetic acid in the urine. Grandjean et al. (1955) reported that workers, most of whom were exposed to 20 to 40 ppm TCE, excreted about 8% of inhaled TCE as trichloroacetic acid in a ratio of 3:1 (3 mg/L trichloroacetic acid in the urine to 1 ppm TCE in the urine to 1 ppm TCE in the air). This ratio was larger in younger people (6:1) than in the older people (2:1).

Results from two experiments described below indicate that there may be a variation in the urinary excretion of TCE metabolites depending on the sex of the subject. More specifically, there may be a sex difference in human metabolism of TCE. There is not enough evidence, however, to substantiate this theory.

Nomiyama (1971) exposed five male and five female students to between 250 and 380 ppm TCE for 160 minutes. Males and females excreted different amounts of trichloroacetic acid and trichloroethanol during the first 24 hours after exposure. Females excreted more trichloroacetic acid in their urine than did males, while males excreted twice as much trichloroethanol as females. Of the retained TCE in males, 32.6% was excreted as trichloroacetic acid and 48.6% as trichloroethanol, whereas in females, 49.3% of retained TCE was excreted as trichloroacetic acid and 42.7% as trichloroethanol.

Similar results were obtained by Kimmerle and Eben (1973b). After exposing eight volunteers (four male and four female) to either 44 ± 4 or 50 ± 7 ppm of TCE for four hours, a difference in the amount of excretion products was noted. Females showed a higher excretion of trichloroacetic acid than did males. No other differences between sexes were observed in urinary excretion levels or concentrations of TCE and trichloroethanol in the blood.

Four male volunteers inhaled 70 and 140 ppm TCE for four hours during exercise and at rest. Monster et al. (1976) reported that exercise increased the quantity of TCE inhaled, but not the distribution of TCE metabolites. Analysis accounted for 67% of the dose: 10% unchanged from lungs, 39% trichloroethanol plus 18% trichloroacetic acid in the urine.

E. Storage--Biological Half-life

Many articles have been published on the biological half-life ($T_{1/2}$) of TCE and its metabolites in humans. Ikeda and Imamura (1973) collected and summarized these previous citations of biological half-lives; an expanded version of these citations is presented in Table III-10. Additional

Table III-10 Biological Half-Life of Metabolites in the Urine of Human Subjects Exposed to Vapors of Trichloroethylene

Group affected	Number of people	Sex	Exposure load and time	Biological Half-Life (hours)			References
				Total Trichloro-compounds	Trichloroethanol	Trichloroacetic acid	
Factory workers	6	M	10 to 150 ppm for 4 hr, 1 or 2 times/mo	42.7 ± 4.5[a] (37.3 ± 6.2)	-- --	-- --	Ikeda and Imamura (1973)
	6	M	5 to 170 ppm for 2 hr, 1 or 2 times/mo	48.4 ± 11.7 (47.5 ± 7.7)			Ikeda and Imamura (1973)
	6	M	Intermittently exposed to 200 ppm, 5 days/week	26.1 ± 4.8) (22.7 ± 4.6)	15.1 ± 2.2 (14.2 ± 2.3)	39.7 ± 8.7 (36.5 ± 17.3)	Ikeda and Imamura (1973)
	6	M	20 to 40 ppm for 8 hr/day for 5 days/week	33.7 ± 6.8 (26.9 ± 5.0)			Ikeda and Imamura (1973)
	6	F	Intermittently exposed to 50 ppm, 5 days/week	50.7 ± 7.7 (38.3 ± 7.5)	42.7 ± 9.1 (12.6 ± 8.9)	57.6 ± 19.8 (50.9 ± 22.6)	Ikeda and Imamura (1973)
Volunteers	2	F	186 ppm for 5 hr	50.3	29.2	55.3	Bartonicek (1962)[b]
	5	M	250 to 380 ppm for 160 min	31.4	19.0	38.0	Nomiyama and Nomiyama (1971)
	5	F	250 to 380 ppm	36.1	25.8	36.1	Nomiyama and Nomiyama (1971)

Table III-10 - continued

Group affected	Number of people	Sex	Exposure load and time	Biological Half-Life (hours)			References
				Total Trichloro-compounds	Trichloroethanol	Trichloroacetic acid	
Volunteers	5	M	170 ppm for 7 hr	35.8	--	--	Ogata et al. (1971)[b]
	4	M	170 ppm for 3 hr	48.6	--	--	Ogata et al. (1971)[b]
	5	M,F	50 ppm for 6 hr	--	12.0	100.0	Muller et al. (1972)[b]
Addict	1	M	--	72.6 (95.1)	49.7 (49.8)	72.6 (95.0)	Ikeda et al. (1971)[b]

[a] Values are mean ± SE calculated from metabolite concentration corrected for a specific gravity of urine of 1.016, together with those corrected for creatinine concentration in parenthesis.
[b] Values are calculated by the present authors from results of referred authors.

studies on the half-lives in the urine, not cited by Ikeda and Imamura, have been collected and added.

Ikeda and Imamura noted a wide variance in biological half-lives (26 to 51 hours) of total trichloro-compounds in urine of factory workers exposed to TCE (Table III-4). There appears to be no correlation between the number of exposures and the variance in biological half-lives. Ikeda and Imamura observed, however, that the total mean value calculated was about 41 hours. This value closely correlates to the experimental values of half-lives in subjects not previously exposed to TCE vapors.

Two other observations based on data from Table III-10 were made by Ikeda and Imamura. First, no sex-related differences were observed in the half-lives of total trichloro-compounds; second, the half-life in an "addicted" patient was higher than those in the factory workers.

Few data have been published on the biological half-lives of TCE in the blood. Table III-11 summarizes the biological half-lives of metabolites of TCE in the blood of human subjects exposed occupationally to TCE vapors.

The biological half-life in the serum and urine of rabbits was reported by Ogata and Saeki (1974) (Table III-12). Results show that, except for TCE and chloral hydrate, the

Table III-11 Biological Half-Life of Metabolites in the Blood of Human Subjects Exposed Occupationally or Experimentally to Vapors of Trichloroethylene

Compound	Groups	Type of exposure	Biological half-life (hours)			Reference
			TTC[a]	TCE[b]	TCA[c]	
Trichloroethylene	Volunteers (5 subjects)	Experimental	--	12[d]	--	Ertle et al. (1972)
	Volunteers (5 subjects)	Experimental	--	13.3[e] 12.4[f]	85.6[e] 99.0[f]	Muller et al. (1974)

[a] Total trichloro-compounds.
[b] Trichloroethylene.
[c] Trichloroacetic acid.
[d] Six hours/day for five days at either 50, 100 or 250 ppm for 12 min/hour (high peak concentration, average 50 ppm).
[e] 100 ppm trichloroethylene, 6 hours/day for 10 days.
[f] 500 ppm TCE, 6 hours/day for 5 days.

Table III-12 Biological Half-Life of TCE and Metabolites in Rabbits[a]

	Half-life (hours)	
Compound	Urine	Serum
Trichloroethylene	--	3.8
Chloral hydrate	--	6.4
Free trichloroethanol	30.5	8.4
Total trichloroethanol	38.0	8.5
Conjugate trichloroethanol	42.0	8.5
Monochloroacetic acid	36.0	14.0
Trichloroacetic acid	43.5	18.5

[a] Rabbits were given 13 mol/kg TCE orally.

From Ogata and Saeki (1974).

half-lives of metabolites in urine are longer than those in serum.

Four subjects were repeatedly exposed to TCE for four hours/day for five days at 50 ppm (48 ± 3 ppm, (Kimmerle and Eben 1973a). It was noted that trichloroethanol could be detected in the human blood up to four days following a single exposure to 50 ppm.

F. Summary and Conclusion

Limited information on the quantitative absorption of TCE via ingestion is available. About 95% to 98% of a single oral dose of TCE was absorbed from the G.I. tract in rats and mice. The extent of absorption by the inhalation route has been reported to be between 51% and 64%. This appears to be misleading, since it is reasonable to believe that at a given concentration of TCE in air, equilibrium between the concentration in air and the concentration in blood would be established. After equilibrium has been established, absorption has been demonstrated to be dependent upon the disposition and metabolism of the chemical. Trichloroethylene has been reported to distribute in the tissues according to their fat contents. It has been determined that TCE crosses the placental barrier, and it has been detected in fetal blood.

Trichloroethylene may be biotransformed in the mammalian system via the formation of an epoxide or an oxygenated TCE-cytochrome P-450 intermediate. The metabolites identified include trichloroacetaldehyde, trichloroacetic acid, dichloroacetic acid, monochloroacetic acid, trichloroethanol, trichloroethanol glucuronide, oxalic acid and N-(hydroxyacetyl)-aminoethanol. In general, the metabolites reported in the animal systems were qualitatively similar to those found in humans.

It has been determined that TCE and its metabolites are eliminated in urine, by exhalation and, to a lesser degree, in sweat, feces and saliva. Urinary excretion of trichloroethanol and trichloroacetic acid appeared to be dose-dependent: the higher the dose, the larger the amount of these metabolites excreted in the urine. Trichloroacetic acid has been reported to bind with plasma protein. On repeated exposure, this metabolite may stay in the body for a long time.

V. ACUTE AND CHRONIC HEALTH EFFECTS IN ANIMALS

A. Hepatotoxicity

Stott et al. (1982) studied hepatotoxicity in male $B_6C_3F_1$ mice receiving 0, 250, 500, 1,200 or 2,400 mg TCE/kg/day by gavage on five days/week for three weeks. There were 10 to 12 mice per dose group. A corn oil vehicle was used. A subcutaneous injection of (6-^{3}H) Trymidine [(^{3}H)dT] was administered three to four hours after dosing on the four days preceding completion of TCE administration. The mice were sacrificed and hepatic tissues were microscopically examined for histopathological changes. The DNA synthesis indicated by (^{3}H)dT incorporation in hepatic tissue was detected by liquid scintillation counting. Histopathological changes in hepatic tissues were observed at all dose levels (except in controls) and appeared to be dose related. In the low dose groups (250 and 500 mg/kg/day), there were slight increases in cytoplasmic eosinophilic staining of centrilobular hepatocytes which indicated glycogen depletion and proliferation of smooth endoplasmic reticulum. The hepatocellular swelling was prominant in the 1,200 mg/kg/day group. Severe centrilobular hepatocyte swelling, giant cell inflamation and mineralized cells were evident in the 2,400 mg/kg/day dose group. The mineralized cells were indicative of individual cell necrosis. There was increased hepatic DNA synthesis (indicative of a regenerative process), although it was not statistically significant.

Tucker et al. (1982) exposed male and female CD-1 mice to TCE in drinking water at concentrations of 0, 100, 1,000, 2,500 and 5,000 mg/L for six months. One percent emulphor was used to dissolve the TCE in deionized water. There were 140 mice of each sex in each TCE-exposed group and 260 mice of each sex in the vehicle control group. The no-treatment control group (140 mice of each sex) received deionized water. Groups of mice were sacrificed at four-months and six-months of exposure. Gross pathological examinations detected abnormalities including pale, spotty and granular livers in all TCE-exposed groups. Detailed data on histopathology were not clearly reported by the authors. Granular livers and fatty infiltration were observed in about 3% (2/58) of the mice at four-months exposure and 19% (11/59) of the mice at six-months exposure. The liver was enlarged in males exposed to 1,000 to 5,000 mg/L and in females at 5,000 mg/L. Pale and spotty livers were infrequent in the vehicle and no-treatment control groups.

Several inhalation studies have provided observations on the hepatotoxic effects of TCE after single or multiple exposures. Kylin et al. (1962) compared the hepatotoxicity of TCE and tetrachloroethylene. Mice were given a single four-hour exposure by inhalation. The animals were sacrificed on the third day, and the livers were analyzed for fat by histological examination and by acetone-hexane extraction. In addition, activity of serum ornithine carbamyl transferase was determined. At a concentration level of 6,400 ppm, TCE produced no significant damage to the liver. In this study, TCE was the least hepatotoxic, whereas chloroform was the most hepatotoxic. Similar results were obtained by Plaa et al. (1958) and Gehring (1965) when animals were exposed to halogenated hydrocarbon solvents by subcutaneous injection and by inhalation. The results of these workers indicate that the halogenated hydrocarbon solvents, in order of their decreasing capacity to cause liver dysfunction, rank: carbon tetrachloride, chloroform, 1,1,2-trichloroethane, tetrachloroethylene, TCE 1,1,1-trichloroethane.

Multiple inhalation exposure studies have been reported utilizing mice, rats and dogs. Seifter (1944) observed degeneration of liver parenchyma cells in dogs that were exposed either to 750 ppm TCE for 8 hours/day, 6 days/week, for 3 weeks or to 500 to 750 ppm TCE for 6 hours/day, 5 days/ week, for 8 weeks. Slight fatty infiltration of the liver of mice was detected by Kylin et al. (1965). Female albino mice were exposed to 1,600 ppm TCE by inhalation for four hours daily, six days/week, over periods of one, two, four and eight weeks. The increase in liver fat content was detectable after one week of exposure; subsequently, the liver fat showed no further increase. In terms of fatty degeneration, the authors noticed that tetrachlorethylene was approximately 1/10 times less toxic than TCE. Male Wistar II rats inhaling 55 ppm TCE for 14 weeks exhibited elevated liver weights, but did not show pathological changes as measured by hematological examinations, liver function tests, renal function tests and blood glucose (Kimmerle and Eben 1973a). Four animal species, rabbits, guinea pigs, rats and monkeys, were exposed to 100 to 3,000 ppm TCE vapor for seven hours daily, five days/week, for periods of up to approximately six months by Adams et al. (1951). Rats exposed to 300 to 3,000 ppm TCE for a period of 36 days (total of 27 exposures) showed an increase in liver and kidney weights. Histopathological examination of the tissues, however, failed to reveal any abnormality in male rats, although some female rats showed fat vacuoles in the cytoplasm of the liver. Rats exposed to 200 ppm TCE for 205 days (total of 151 exposures) showed no significant abnormality compared to the controls. The authors concluded that the maximum concentrations without adverse effects were as follows: monkey, 400 ppm; rat and rabbit, 200 ppm; guinea pig, 100 ppm.

B. Nephrotoxicity

There are conflicting reports in the literature regarding renal damage resulting from parenteral and inhalation exposure of animals to TCE. A long-term inhalation study on rats, guinea pigs, dogs, rabbits and monkeys by Prendergast et al. (1967) showed that no nephrotoxicity occurred at continuous concentrations of 35 ppm (189 mg/m^3) for 90 days and 730 ppm (3,825 mg/m^3) for 8 hours/day, 5 days/week, for 6 weeks.

Plaa and Larson (1965) found that after injecting mice intraperitoneally with 0.6 mL/kg of TCE, no renal toxicity was observed. The acute nephrotoxic properties were studied using phenol sulphthalein excretion, the presence of proteinuria and glucosuria and histopathology. When Bartonicek and Soucek (1959) injected six rabbits (average weight of 4.2 kg) intramuscularly with 33 to 55 g of TCE over a period of 55 to 100 days, however, two of the rabbits died from renal failure.

C. Nervous System

Because of its effects on the nervous system, TCE has been used as a general anesthetic agent. Studies performed as early as 1944 give information concerning the blood concentration of TCE for lethal as well as anesthetic effects. Dogs, rabbits, guinea pigs and cats were administered TCE by inhalation. Blood levels were determined at death and at anesthesia stages. The lethal blood TCE concentration in dogs was found to be 100 to 110 mg/100 mL blood. At the anesthetic stage, TCE blood levels were 24 to 37, 23 to 28, 14 to 18 and 25 to 32 mg/100 mL blood for dogs, rabbits, guinea pigs and cats, respectively. The blood-brain ratio at anesthetic dosages was approximately 1:2 for both guinea pigs and dogs (Kulkarni 1944).

Histopathological changes have been observed on acute and long-term exposure of animals to TCE. A single exposure of dogs to 30,000 ppm TCE in air resulted in death within 20 minutes. No obvious changes were found in the nervous system. In a more long-term experiment, the animals were subjected to TCE concentrations ranging from 500 to 3,000 ppm for periods of from 2 to 8 hours/day, often for 5 days/week. The total exposure period was between 60 and 162 hours. The exposure appeared to have selectively destroyed the Purkinje layer of the cerebellum. The cerebral hemispheres showed mild changes; scattered cortical neurons became swollen or pyknotic, and the white matter of the myelin developed a mild focal swelling (Baker 1958). Bartonicek and Brun (1970) injected TCE intramuscularly in female rabbits and observed moderate neurological changes in the exposed animals. The dosage regimen included subacute exposure for 29 days. Animals were injected with 2.47 g/kg body weight three times a week. For the chronic exposure experiment, animals were injected intramuscularly with 1.62 g/kg twice a week for 41 to 247 days. The rabbits were sacrificed at different times during the test, and the brains were examined histologically and histochemically for any pathological change. Round cell infiltration around blood vessels and in the parenchyma occurred in all animals in the subacute study and in one animal in the chronic experiment, but not in the controls. Disappearance of Purkinje cells and basket cells was definitely shown only in the chronic experiment.

Grandjean (1960) exposed male rats to 200 and 800 ppm TCE vapor for 4 to 11 weeks. The rats were subjected to a single three-hour TCE exposure just before testing. After the exposure, trained rats, responding to signals, climbed up a rope to reach a feeding trough, where they found a small dextrose pellet as a reward. The results indicate that the increase in the number of spontaneous climbs after exposure to the solvent was significant in comparison with the control tests. The observed effect was not dose-dependent. The author concluded that TCE, in the doses studied, modified the psychological equilibrium of rats by increasing excitability. In his 1963 report, Grandjean (1963) described the effect of TCE vapors on the swimming performance and motor activity of rats. The animals were exposed for six hours, and swimming tests were performed 5 to 15 minutes later. At 400 ppm, TCE only retarded the performance (in a manner barely significant) of the rats swimming with an additional load, while 800 ppm adversely affected the performance of the rats, both with and without the load, in a significant manner. One hour after termination of exposure, no significant changes in the swimming times could be observed.

D. Cardiovascular Effects

Trichloroethylene caused depression in myocardial contractility (Aviado et al. 1976). The minimum inhaled concentration of 500 ppm caused a depression in the myocardial contractility in dogs. Transitory arrythmia was observed in the isolated guinea pig heart at a concentration of 5,300 ppm.

E. Reproductive and Teratogenic Effects

Zenick et al. (1984) studied reproductive effects of TCE in male Long-Evans rats (10 animals/dose group, 100 days of age) intubated with 0, 10, 100 or 1,000 mg/kg/day for five days/week for six weeks. Copulatory behaviors were scored and semen was evaluated for sperm count, motility and morphology during four weeks pre-exposure, at one- and six-weeks of exposure and at four-weeks post-exposure. Testosterone levels in blood were also determined at these intervals. At six-weeks exposure, three rats from each dose group were randomly selected and sacrificed. The tissue and blood levels of TCE and major metabolites (trichloroethanol and trichloroacetic acid) were analyzed by gas chromotography. The remainder of the rats were sacrificed at four weeks post-exposure.

No statistically significant TCE-related effects on sperm count, motility or morphology were detected within or among groups at any phase of the study. Plasma testosterone levels were also unaffected at six-weeks exposure and four-weeks post-exposure. Copulatory behavior was impaired by administration of 1,000 mg/kg/day during the initial four weeks of exposure; although copulatory behavior returned to normal by week five. Impairment was characterized by protacted mating, neglect of females, incomplete contact and lack of vigor. The authors suggest that the observed alterations of copulatory behavior may result from the narcotic action of TCE and that the absense of the effects at week-five may be due to acquired tolerance to TCE's pharmacological effects. Tissue analyses detected that TCE and its metabolites accumulated in male reproductive organs; however, spermatotoxicity was not observed.

Manson et al. (1984) studied effects of oral exposures to TCE on the female reproductive function in rats. Groups of 23 female Long-Evans hooded rats (80 to 100 days old) were administered 0, 10, 100 or 1,000 mg/kg/day of TCE in a corn oil vehicle for two weeks premating, one week of mating and three weeks of pregnancy. The TCE doses were administered five days/week during premating and mating periods then seven days/week upon insemination. The number of mating trials to insemination was measured as the fertility index. Failure to become inseminated within eight days was considered a sign of infertility. Exposure to TCE did not adversely effect the estrus cycle length or fertility in any dose group. Five dams in the highest dose group died and weight gain in this group was significantly depressed throughout the treatment periods. Neonatal mortality was significantly ($P < 0.001$) increased in offspring from the 1,000 mg/kg/day dose group compared to all other dose groups and controls. The majority of deaths occurred among female offspring at time of birth. No major malformations were detected upon gross examination of the pups. The maternal weight gain during pregnancy, litter size at birth and neonatal survival were not impaired in the 10 or 100 mg/kg/day dose groups. The authors concluded that oral exposure to TCE below the dose causing limiting maternal toxicity had no affect on female fertility or pregnancy outcome.

Trichloroethylene does not appear to be teratogenic in animals. Pregnant rats and mice were exposed to 300 ppm TCE vapor for seven hours daily on days 6 to 15 of gestation. This exposure resulted in a slight, but statistically significant, reduction in mean body weights of maternal rats, but not of mice, during and/or following exposure. No teratogenic abnormalities were observed in either species (Schwetz et al. 1975).

In another study, Dorfmueller et al. (1979) exposed female Long-Evans hooded rats by inhalation to TCE at a concentration of 1,800 ± 200 ppm (9,810 ± 1,090 mg/m^3) for two weeks before mating and during the first 20 days of pregnancy. Rats were measured for changes in body weight every four days. Fetuses were weighed and examined for skeletal and soft tissue anomalies. Postnatal behavioral changes were examined by activity measurements with the aid of electronic motility meters. The most frequently observed skeletal anomaly was incomplete ossification of sternum, which is indicative of delayed skeletal ossification rather than being a true malformation. No overt maternal toxicity, embryotoxicity or teratogenicity was observed as a result of TCE treatment.

F. Mutagenic Effects

There have been a number of recent studies using various assay techniques to determine the mutagenic potential of TCE. Current results are tabulated in Table V-1, with both positive and negative results depending on the test system and whether or not the system was metabolically activated.

The bacterial mutagenesis system is most commonly used as a screening technique to determine the mutagenic and carcinogenic potential of chemicals. Trichloroethylene was found to be mutagenic in *Salmonella typhimurium* strains and the E. *coli* K 12 strain, utilizing liver microsomes for activation (Greim et al. 1975, 1977). Bartsch et al. (1979) used S-9 fractions from liver specimens instead of microsomes for activation in the mutagenesis test. These authors reported that TCE was marginally mutagenic. Waskell (1978) reported that TCE was non-mutagenic in the Ames test system with

Table V-1 Mutagenicity Testing--Trichloroethylene

Test system	Reaction tested	Result	Reference
Microbial			
Salmonella typhimurium	Gene mutation	Mutagenic in activated system	Greim et al. (1975)
Salmonella typhimurium	Gene mutation	Mutagenic in activated system	Bartsch et al. (1979)
Salmonella typhimurium	Gene mutation	Non-mutagenic	Waskell (1978)
Escherichia coli K-12	Gene mutation	Mutagenic in activated system	Greim et al. (1977)
Saccharomyces cerevisiae	Mitotic gene conversion	Positive	Bronzetti et al. (1978)
Saccharomyces cerevisiae SV185-14C	Gene mutation Frameshift mutation	Positive	Shahin and vonBorstel (1977)
Animal			
Fischer rat embryo	Cellular transmation	Positive	Price et al. (1978)

activation. The negative responses obtained by later researchers cannot be explained at the present time.

Saccharomyces cerevisiae (yeast) and Fischer rat embryo have also been used to study mutagenic response. After activation with liver microsomal fractions, TCE was found to be mutagenic *S. cerevisiae* strains D_4, D_7 and XV185-14C (Bronzetti et al. 1978, Shahin and vonBorstel 1977). Price et al. (1978) tested TCE for *in vitro* cell transforming potential in a Fischer rat embryo system (F1706). The transformed cells grew in a semi-solid agar and produced undifferentiated fibrosarcomas when inoculated into newborn Fischer rats.

G. Carcinogenic Effects

The National Cancer Institute (NCI 1976) conducted a study to delineate the carcinogenic potential of TCE. Using both sexes of Osborne-Mendel rats and $B_6C_3F_1$ mice. For rats, the initial doses were 1,300 and 650 mg/kg body weight. The dosages were changed, based upon survival and body weight data, so that "time-weighted" average doses were 549 and 1,097 mg/kg for both male and female animals. The time-weighted average daily doses were 1,169 and 2,339 mg/kg for male mice and 869 and 1,739 mg/kg for female mice. Animals were exposed to the compound by oral gavage, five times per week for 78 weeks, and observed until sacrifice at 110 weeks for rats and 90 weeks for mice. A complete necropsy and a microscopic evaluation were conducted on all the animals (except for seven, out of the original 480, which died at unscheduled times).

No significant difference was noted in neoplasms of experimental and control groups of rats. In both male and female mice, however, the higher dose induced primary malignant tumors in the liver. For males, 26 of 50 mice receiving the low dosage and 31 of 48 mice receiving the high dosage developed hepatocellular carcinomas, while only 1 of 20 controls showed neoplasms. In female mice, 4 of 50 mice receiving the low dosage and 11 of 47 mice receiving the high dosage developed neoplasms, as compared to zero out of 20 controls. The results of this experiment indicate that TCE induced a hepatocellular carcinoma response in mice. Under the conditions of this experiment, the rats did not elicit a carcinogenic response.

In the NCI (1976) study cited above, the TCE used in the test was later found to contain epichlorohydrin, a carcinogen. Therefore, NCI repeated the bioassay with epichlorohydrin-free TCE. Rats (F344/N) and mice ($B_6C_3F_1$) of both sexes were used. Trichloroethylene was mixed with corn oil and administered by gavage five times per week for 103 weeks. Rats received dosages of 500 and 1,000 mg/kg. These dose levels were lower than the initial doses used in the earlier bioassay in Osborne-Mendel rats (650 and 1,300 mg/kg for both sexes).

The dosage level used in the mice, 1,000 mg/kg for both sexes, was also lower than in the earlier study.

Trichloroethylene was not found to be carcinogenic in female F344/N rats. The experiment with male rats was considered to be inadequate, since these rats received dose levels of TCE which exceeded the maximum tolerated dose. Trichloroethylene was demonstrated to be carcinogenic in both sexes of $B_6C_3F_1$ mice, producing hepatocellular carcinomas.

In another study by Rudali (1967), oral doses of TCE were administered by gavage to 28 NLC mice (age not specified). Dosages of 0.1 mL of a 40% solution of TCE in oil were administered twice weekly for an unspecified time. No liver lesions or hepatomas were observed. In a similar set of experiments, chloroform was slightly oncogenic.

Henschler et al. (1984) studied tumorigenicity of chronic oral administration of 2,400 mg TCE/kg/day in male and 1,800 mg TCE/kg/day in female ICR/HA Swiss mice. Groups of 50 mice of each sex were administered corn oil (controls) or TCE in corn oil with and without addition of stabilizers [0.0015% triethanolamine, 0.8% epichlorohydrin (EPC), 0.8% 1,2-epoxybutane (EB) or a mixture of 0.25% EPC and 0.25% EB] by gavage for five days/week for 18 months. The daily dosing was intermittently interrupted and the TCE doses were reduced by 50% after 40 weeks due to non-specific toxicity and poor condition of the animals. Dosing in all groups was stopped during weeks 35 to 40, 65 and 69 to 78. The overall observation period was 24 months. Deceased animals were immediately autopsied including gross and microsopic examinations of all organs. The survivors were sacrificed and subjected to similar examinations. The only statistically significant ($P < 0.05$) increased tumor incidence detected was that of forestomach papillomas and carcinomas in groups receiving TCE with EPC, EB or the mixture of EPC and EB stabilizers. The group dosed with the amine-based-stabilized-TCE had no significantly increased tumor incidences in the forestomach or any other site. Thus, the authors concluded that there was no indication of tumorigenic potential of pure amine-base-stabilized TCE whereas addition of EPC or the mixture of EPC and EB stabilizers resulted in forestomach cancer in both sexes.

Van Duuren et al. (1979) studied the carcinogenicity of TCE administered by gavage, by dermal application or by subcutaneous injection to groups of ICR/HA Swiss Mice. A dose of 0.5 mg TCE/mouse in 0.1 mL trioctanoin was administered by gavage once weekly for 89 weeks. There were 30 mice of each sex in the TCE treatment group and in the vehicle (0.1 mL trioctanoin/week) control group. The no treatment control group contained 60 male and 100 female mice. The animals were examined daily and those in poor health or with large tumor masses were sacrificed. All animals were completely autopsied upon death or termination of the experiment. Histopathological examinations were performed on all abnormal-appearing tissues and organs. Tissue sections from the stomach, liver and kidneys were routinely examined for histopathology. The incidence of forestomach tumors (2/30 males and 1/30 females) was not significantly ($P > 0.05$) higher than those of controls (8/60 males and 5/100 females).

Trichloroethylene was not an active skin tumor initiator in the two-stage carcinogenisis assay. In this initiation-promotion assay, a single dose of 1.0 mg TCE in 0.1 mL acetone was applied to the shaved dorsal skin of each mouse (30 females) followed by 2.5 micrograms PMA (a known promotor) in 0.1 mL acetone three times/week. The positive controls (120 females) were treated with PMA only. The controls (100 females) received no treatment.

In another assay, repeated dermal application of TCE produced no tumors. A dose of 1.0 mg TCE in 0.1 mL acetone was applied three times/week for 83 weeks to the shaved dorsal skin of 30 female mice. There were 30 mice in the vehicle control group and 100 mice in the no treatment control group.

In the subcutaneous injection study, a dose of 0.5 mg TCE in 0.05 mL trioctanoin was administered to 30 female mice in their left flank, once/week for 89 weeks. The vehicle control (0.05 mL trioctanoin) and the water control (0.05 mL water) groups (30 females each) received similar injections. The no treatment control group contained 100 females. Routine sections of the livers and injection sites were examined at autopsy for histopathology. No local sarcomas were detected in the TCE treated group or the controls. Thus, Van Duuren et al. (1979) concluded no indication of carcinogicity was evident in mice administered TCE by the various routes under these experimental conditions.

Henschler et al. (1980) studied the effect of chronic inhalation of TCE on the tumor incidence in NMRI mice, WIST rats and Syrian hamsters. Groups of 30 males and 30 females of each species were exposed by inhalation to 0, 100 or 500 ppm TCE for six hours/day for five days/week for 18 months. The TCE was highly pure (> 99.99%) and stabilized with 0.0015% triethanolamine. A necropsy and microscopic examination was performed on all deceased animals. At 30 months (for mice and hamsters) and 36 months (for rats), survivors were sacrificed and microscopically examined. No statistically significant increased tumor incidence was observed in any species or dose group except that for malignant lymphomas in female mice. The incidences of lymphomas in the 100 ppm group (17/30) and the 500 ppm group (18/28) were significantly ($P < 0.05$) higher than in controls (9/29).

Fuduka et al. (1983) examined the effect of chronic inhalation of reagent grade TCE (99.8% pure) on tumor incidence in female ICR mice and Sprague-Dawley rats. The impurities in the vapor phase were 0.128% carbon tetrachloride, 0.019% benzene, 0.019% epichlorohydrin and 0.010% trichloroethane. Groups of 49 to 51 animals of each species were exposed to 0, 50, 150 or 450 ppm TCE for seven hours/day on five days/week for up to 104 weeks. Survivors were sacrificed at three-weeks post-exposure (i.e. 107 weeks after initiation of exposure) and complete histopathological examinations were performed. In mice, the incidences of pulmonary adenocarcinomas in the 150 ppm group (8/50) and the 450 ppm group (7/46) were significantly ($P < 0.05$) higher than that of controls (1/49). The average number of lung tumors per mouse in groups exposed to 150 ppm TCE (0.46) and 450 ppm TCE (0.39) were more than 3 times that of controls (0.12). No significant differences in incidences of other tumor types, tumors of specific organs by site of origin and numbers of animals affected were observed in either species.

H. Synergistic and/or Antagonistic Responses

There are a few reports which suggest interactions between TCE and other drugs/chemicals when given concurrently and/or in sequence. The interactions have been reported at very high dose levels given for short durations. Interaction studies for longer durations are not available. Therefore, the information cited below should not be used for making any adjustment to the standard.

Cornish and Adefuin (1966) found that the hepatotoxic response was markedly potentiated by prior ingestion of ethanol. These workers exposed rats to TCE (10,000 ppm) for 1.5 hours. Pretreatment of rats with phenobarbital (50 mg/kg, 2.p.) or 3-methylchloanthrene (40 mg/kg) increased TCE-induced liver damage as indicated by serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (Carlson 1974). The possible mechanism behind these observations has been described in Section VII.

I. Summary and Conclusion

Trichloroethylene has been reported to adversely affect the livers of exposed animals. For acute exposures, the halogenated hydrocarbon solvents, in order of decreasing capacity to cause liver dysfunction, were ranked: carbon tetrachloride, chloroform, 1,1,2-trichloroethane, TCE, 1,1,1-trichloroethane. Animal species which have been reported to respond to the toxic effects of TCE on liver are mice, rats, rabbits, guinea pigs, dogs and monkeys; however, which of the species is the most sensitive has not been precisely determined.

Chronic exposure of animals to TCE induced nephrotoxic responses. At very high dose levels, TCE was demonstrated to produce anesthesia. At the anesthetic stage, TCE blood levels were reported to be 24 to 37, 23 to 28, 14 to 18 and 25 to 32 mg/mL blood for dogs, rabbits, guinea pigs and cats, respectively.

Oral administration of 1,000 mg TCE/kg/day for six weeks impaired copulatory behavior in male mice but spermatotoxicity was not evident. Oral administration of 1,000 mg TCE/kg/day to female mice prior to and during pregnancy had no effect on estrus cycle and fertility. Neonatal mortality was significantly increased although major malformations in offspring were not detected. TCE was not found to be teratogenic when administered by inhalation. It was considered to be a weak mutagen, as indicated by microbial test systems.

In a repeat study with epichlorohydrin-free TCE, the NCI found that TCE was carcinogenic in both sexes of $B_6C_3F_1$ mice. The experiment with male rats was considered to be inadequate to establish carcinogenicity. There was no indication of tumorigenic potential of amine-based stabilized TCE in ICR/HA Swiss mice in a chronic oral study. The significance of this study is uncertain since dosing was intermittently interrupted and dose levels were decreased due to non-specific toxicity and poor condition of the animals. Another gavage study in ICR/HA Swiss mice found no increased incidence of tumors in the stomach, kidney or liver. Trichloroethylene was not carcinogenic in ICR/HA Swiss mice when administered by subcutaneous injection or skin painting. Inhalation studies found an increased incidence of malignant lymphomas in female NMRI mice and pulmonary adenocarcinomas in female ICR mice; the tumor incidence in WIST rats, Sprague-Dawley rats and Syrian hamsters was not affected by TCE inhalation.

Trichloroethylene has been reported to interact with ingested ethanol. This information cannot be used to derive a standard for TCE in drinking water because the duration of exposure was too short. In addition, it is reasonable to believe that the interaction was dose-dependent and that, at lower concentrations, the interaction may not exist.

VI. HUMAN HEALTH EFFECTS

A. Acute Exposure

The following section includes information concerning the acute effects of TCE exposure by either ingestion or inhalation. Special attention has been given to the dosages (in mg/kg body weight) which have been reported to produce an effect.

Single oral dosages ranging from 7.6 to 35 g have been reported to elicit clinical symptoms in humans. A four and a half year-old child who ingested an estimated 7.6 g of TCE vomited, became inebriated and lost consciousness within a few minutes, but recovered after four hours (Gibitz and Plochl 1973). Two persons who each consumed 15 to 25 mL (21 to 35 g) of TCE experienced vomiting and abdominal pain, followed by inebriation and transient unconsciousness (Stephens 1945).

Morreale (1975) reported one 56-year-old patient who drank 15 mL TCE and, along with neural intoxication, suffered a myocardial infarct which was attributed to the TCE.

Bernstein (1954) stated that a 19-year-old Marine who underwent TCE anesthesia suffered cardiac arrest (due to an excessive concentration of TCE in the body), but subsequently recovered. In another report, electrocardiographic abnormalities were seen in 15 of 30 patients exposed acutely to high levels to TCE. Arrhythmia was the most frequent effect (Pelka and Markiewicz 1977).

Tomasini (1976) reviewed Italian case histories of TCE-related toxicity. In about one-fourth of a group of 35 patients, cardiac arrhythmia of some degree had occurred after TCE exposure. Accidental, intentional and industrial exposures were included in the population. The TCE levels that produced fatalities ranged from oral introduction of 50 cc pure TCE in a 21-year-old male to a "pitcher" of Trilene in a 38-year-old female. Cardiac histories of the industrial workers were not described, and the quantities of TCE that produced cardiac effects were not reported. The author suggested that the mechanism of cardiotoxicity was depression of normal rhythm which permitted any other ectopic foci present to break the normal myocardial rhythm. It was observed that TCE, as sold, is sometimes a mixture of several chlorinated solvents. The relationship, if any, between specific Italian additives and the cardiac effects described was not further developed.

Depending upon the dose, inhalation of TCE results in mild to severe central nervous system depression. Salvini et al. (1971) observed psychophysiological changes in human volunteers in a controlled inhalation study using TCE at levels as low as 110 ppm for two 4-hour periods. At 200 ppm TCE, Stopps and McLaughlin (1967) noted a slight decline in the performance of subjects that became increasingly pronounced at 300 and 500 ppm.

Industrial accidents provide some information about the toxic effects of TCE; however, these reports do not provide precise dosages. Buxton and Haywood (1967) described four cases of industrial accidents involving TCE. Four workers were required to climb inside tanks containing TCE and scoop out the remaining liquid with buckets. All four workers became ill, and one subsequentely died. In two men who spent less than 30 minutes inside the tanks, the symptoms of TCE intoxication were nausea and headaches. The symptoms observed in a third man who remained inside the tanks for two and a half hours were nausea, diplopia and facial dysplegia. The fourth man, exposed to TCE vapors for the longest time period, died after developing severe multiple cranial nerve palsies 51 days after initial exposure. The authors ascribed the effects to unidentified decomposition products of TCE.

Six women, employed in cleaning optical lenses for binoculars, used their fingers to apply TCE to remove small spots of wax remaining on the lenses. After a few months, they reported difficulty in handling the lenses because they could no longer feel the lenses properly. Examination showed persistent loss of tactile sense, inability to grasp objects between thumb and fingers and loss of motion. Disability lasted for several months (McBirney 1954). No skin damage was noted in any of these cases.

Maloof (1949) reported a worker who became comatose, suffered convulsions and had to be treated for first, second and third degree chemical burns after entering a freshly drained, heated degreasing tank. Upon awakening, the worker complained of blurred and double vision and burning sensation of the skin. He recovered 31 days later. Another worker involved in the incident became unconscious, but regained consciousness almost immediately.

A man employed for one month as a metal degreaser lost his sense of taste; after two months of employment, he developed trigeminal analgesia. Non-recovery of taste and trigeminal sensation was reported ten months later (Mitchell and Parsons-Smith 1969).

Trichloroethylene has been shown to cause hepatic necrosis in man following either inhalation or ingestion (Ossenberg et al. 1972, Chiesura and Corsi 1961). Liver damage does not always occur in TCE intoxication, however. Most occupational studies on man have shown an increase in serum transaminases, which indicates damage to the liver parenchyma (Albahary et al. 1959, Lachnit 1971). These increases were transient and usually disappeared after exposure was terminated.

B. Chronic Exposure

Toxic hepatitis was observed in a patient who had been cleaning a tank in which TCE was used to clean machine parts. Evidence of liver damage was based on rising levels of SGOT, SGPT and lactic dehydrogenase (LDH) (Bauer and Rabens 1974). These levels returned to normal six weeks later. How long this person had been employed or whether he had cleaned more than one tank as part of his regular duties was not indicated.

Milby (1968) reported a case of TCE intoxication in a 39-year-old female employed for two years as a paint-stripping operator. Six months prior to medical attention, she had been assigned to a newer model stripping machine. She showed no signs of liver injury, even though she complained of daily nausea and vomiting, drunkenness, abdominal cramps, flushing, slepiness, loss of appetite and swelling of the eyes, face and hands. Her physician observed a non-specifically abnormal electrocardiogram and excretion of 780 mg trichloroacetic acid/L in her urine on the day of examination. One week later, she excreted 40 mg trichloroacetic acid/L of urine.

Eight workers were exposed to TCE in an electroplating plant for two to three weeks. The concentrations in the workroom ranged from 115 to 384 ppm (627 to 2,093 mg/m^3). Symptoms began almost immediately after exposure and included headaches, muscle and joint pains, nausea, vomiting, loss of appetite, depression, dizziness and narcosis. All eight subjects showed an increased globulin fraction and a decreased albumin fraction. It was concluded that liver damage was present, as indicated by the cephalin cholesterol flocculation test (CCF) and hyperglobulinemia observations (Nomura 1962).

Guyotjeannin and van Steenkiste (1958) reported that 18 workers exposed regularly to TCE showed signs of abnormal lipid metabolism characterized by total lipid content determination, analysis of lipid fractions and unsaturated fatty acid content. There was also an increase in Y-globulins.

Joron et al. (1955) found massive liver necrosis in a patient who had been exposed to TCE vapors previously and then suffered an acute exposure lasting two and a half hours. The patient died more than one month after the last known exposure to TCE.

The toxic effects of TCE on the urinary system in man have not been well defined. Only a few incidences of renal damage due to TCE intoxication have been reported. Acute hepatic and renal damage was reported in three patients with histories of drug abuse. Centrilobular hepatic necrosis was found in one patient (Baerg and Kimberg 1970). These effects were attributed to sniffing Carbona cleaning fluid or Carbona No. 10 special spot remover, which may contain TCE, petroleum solvents and 1,1,1-trichloroethane.

Gutch et al. (1965) reported that a needle biopsy test showed acute tubular degenerative changes in the kidney of a 41-year-old man who had inhaled TCE vapors. The man had been replacing asphalt floor tile in a small, enclosed room (10 x 20 ft.) with a small ventilation opening in one window. Trichloroethylene (99.5% pure) was used as a solvent to clean tile cement. A gallon container of TCE remained open during the cleaning operation, which lasted over two hours. Inhalation exposure was estimated to be between 166 and 3,700 ppm. After leaving work, the man complained of headache, shortness of breath and vomiting. He admitted himself to a hospital five days later and was diagnosed as having acute renal failure. Kidney function returned to normal after a five-week rest. It is important to note that consistent, moderate to heavy use of alcohol had been reported in this case.

Another case of renal failure after accidental oral ingestion was reported by Kleinfeld and Tabershaw (1954). A patient who had ingested liquid TCE developed jaundice and oliguria and died as a result of acute hepato-renal failure. The amount ingested was unknown. The patient had been in good health, was a moderate beer drinker and had consumed several bottles of beer on the morning of the accident.

Cardiac arrhythmia is the most frequent effect of TCE on the heart. The most direct proof that TCE can cause ventricular fibrillation and cardiac arrest is that these changes can be demonstrated on electrocardiograms (ECGs) of subjects who have accidentally ingested TCE. There are also reports of TCE-related deaths due to ventricular fibrillation.

Trichloroethylene is believed to sensitize the heart to epinephrine, resulting in ventricular fibrillation; thus, any form of stress would help induce cardiac sensitization. Anesthetic concentrations of TCE have been shown to cause changes in the ECG that indicate tachycardia and arrhythmias. The ECG changes that occur during TCE anesthesia in man usually cease when exposure is terminated.

Radonov et al. (1973) reviewed the cases of 200,000 women who were given TCE as an analgesic during therapeutic abortions. Seven deaths occurred; the deaths were attributed to cardiac arrest.

Starodubtsev and Ershova (1976) successfully used TCE-air anesthesia in 128 cases of dental surgery at all three levels of stage 1 anesthesia. Electrocardiograms showed no apparent toxicity.

Four deaths due to chronic exposure to TCE were reported by Kleinfeld and Tabershaw (1954). Exposure concentrations were unknown for three of the four cases. In one case, the concentration measured after the final incident was between

200 and 8,000 ppm. All four workers continued to work at their jobs, even though they complained of nausea and vomiting, drowsiness and dizziness. All died within a few hours after leaving the plant. The mechanism of death was considered to be ventricular fibrillation. Autopsies revealed no gross anatomical abnormalities, but toxicological analysis of the tissues revealed the presence of TCE.

C. Epidemiology

Grandjean et al. (1955) examined 50 workers exposed to TCE in degreasing operations in the Swiss mechanical engineering industry. Clinical exams, case histories, trichloroacetic acid analysis of urine and other clinical blood and urine analyses were done. Medical histories and urine samples were taken from an additional 23 workers. The average age of the 50 workers examined clinically was 43 years; length of exposure ranged from one month to 1[illegible] were both open and closed degreasing tanks; air TCE concentrations in 96 samples ranged between 1 and 355 ppm. Trichloroacetic acid (TCA) levels in urine ranged from 8 to 444 mg/L.

These authors found that the air measurements did not adequately reflect exposures because of the great variations in TCE concentration due to ventilation and operating schedules for degreasers. The general health of the men examined was frequently bad; the authors felt that this was related to the pay and the poor standard of living. Although Grandjean et al. (1955) stated they were not acquainted with the normal incidence of disease in Swiss workmen, they did not examine an unexposed control or comparison group. Of greater importance, they noted the following dose-effect relationships. Neurological and vegetative nervous system disorders were more frequent in the men with the longest histories of work exposure. Subjective symptoms were the same, regardless of length of exposure. Subjective symptoms and vegetative and neurological disorders were more frequent in the higher exposure groups, as determined by the amount of trichloroacetic acid in urine. The persons with symptoms of chronic poisoning were from workplaces with measured air concentrations of TCE between 20 and 80 ppm and had between 10 and 250 mg/L TCA in their urine. Finally, 10% of the workers examined (5) showed evidence of slight impairment of liver function, but the authors were not sure that this could be related to TCE exposure.

Bardodej and Vyskocil (1956) examined 75 persons who worked with TCE (12 in dry-cleaning establishments and 55 in degreasing metal parts). Length of exposure varied between 1/2 and 25 years. Air concentration in these plants varied between 0.028 and 3.4 mg TCE/L (5 to 630 ppm). Eight disabled former employees were also followed clinically. Intolerance to alcohol, shivers, giddiness, neurasthenic syndrome with anxiety states, bradycardia and conduction disturbance of the heart muscle were found to be significantly correlated ($P<0.01$) with duration of exposure. The frequencies of lacrimation, reddening of skin, decreased sensitivity of the hands and disturbances of sleep among this mixed group of workers were also significantly correlated with duration of exposure (P<0.05). No control group was observed, and the age and sex distribution in this group of workers was not given in the description of this study.

Takamatsa (1962) studied 50 male and female workers exposed to TCE for approximately two and a half years during degreasing operations in a communicating machine factory. Screening of workers in January and November, 1960, included a questionnaire, blood cell count, blood pressure measurement and analysis of urine for albumin, sugar, urobilinogen and TCA. Urine was collected twice for each worker, once in the morning and once in the afternoon. On the basis of the results, workers were selected for further examination, including fatigue tests. A control group of 48 non-exposed workers was referred to in the paper, but no information on their characteristics was given.

Eighty percent of the air values in January and 70% of those in November fell between 25 and 100 ppm TCE. Variations in air concentrations were related to proximity to the degreasing apparatus and location of air currents. The mean value of urinary TCA was 66 mg/L. Wide variations in TCA occurred, depending again on proximity to main currents of vapor. A majority of the 50 exposed workers had some complaints, including headache, vertigo, diplopia, sleeplessness, fatigue, etc. Thirty-eight percent of the workers had slight or moderate visual disturbances, and 15% had diplopia. Diastolic blood pressure exceeded that of the (unknown) control group by 5 mmHg. No significant differences in blood count were observed. Decreases in albumin concentration and increases in gamma-globulin were observed in exposed workers and were more frequent in those exposed to the highest air concentrations (150 to 250 ppm). Thirty percent of the workers had albumin in the urine, and elevated urobilinogen was found in 36% of workers. Some of the workers reported constriction of the visual field.

Six employees who worked in the degreasing room had urinary TCA values ranging from 370 to 1,000 mg/L; these workers had frequent complaints, but demonstrated few other clinical findings after short-term (7 to 30 days) exposure. In workers with the highest exposures (150 to 250 ppm), subjective complaints included headache, dizziness, giddiness, drunken feeling, flushing of the face, burning throat and fatigue. The level of TCA in the urine was greater than 100 mg/L and increased during the work week. Malfunctions of the liver were observed, as were changes in serum protein fractions. Workers exposed to 50 to 100 ppm complained of headache, burning eyes, flushing of the face and fatigue. Half of these workers had urinary TCA levels exceeding 100 mg/L. Changes in serum protein fractions were observed, and visual disturbances were found in workers exposed for several years. Work efficiency was reduced by the end of the work week. Workers exposed to less than 50 ppm TCE showed no apparent ill effects. Their urinary TCA levels were less than 50 mg/L.

Lilis et al. (1969) examined 70 workers in a Rumanian semiconductor manufacturing plant. Of these workers, 83% were less than 30 years old, and 74% were women. Duration of exposure was less than two years for 55% of the workers and not more than six years for the remaining workers. The authors collected 214 air samples at the workplaces; of these, 40% exceeded 50 mg/m^3, and 12% exceeded 200 mg/m^3. Trichloroacetic acid concentrations in the urine of these workers exceeded 20 mg/L in 46% of the cases, 40 mg/L in 24% of the cases and 100 mg/L in 7.3% of the cases. Examination included a detailed occupational history questionnaire and elicited information on the onset and occurrence of persistent symptoms. The physical examination paid special attention to the nervous system, heart and vessels and liver and included ECGs and a test for the presence of a metabolite of catecholamine in the urine. Of the workers examined, 75% reported prenarcotic symptoms during the workshift, including dizziness (88%), headache (74%), nausea (43%), euphoria (31%), palpitation (29%), disturbances of vision (21%) and sleepiness at end of shift (29%). These symptoms appeared daily in more than one-third of the examined workers. Persistent symptoms of the pseudo-neurasthenic type appeared after several months of exposure, including fatigue, headache, irritability, anxiety, loss of appetite and alcohol intolerance, along with such signs of autonomic system inbalance as excessive sweating, palpitation and nausea. Physical exams showed few abnormalities. Moderate tachycardia was found in 14% of the cases. Electrocardiographic abnormalities did not appear to be related to toxic exposure, but were said to be similar in frequency to every population group.

Systemic hemodynamic parameters were compared in 44 exposed workers and ten unexposed controls who were similar in age and sex. In the exposed workers, significantly raised mean values of stroke volume, cardiac output, cardiac index and heart work were found and were considered to be signs of epinephrine-type hypersympathicotonia. In support of this, the authors reported that urinary vanillinemandelic acid (3-methoxy-4-hydroxymandelic acid) values differed significantly between exposed workers and controls using a student test ($P < 0.01$).

The scanty description of the control group and its participation in only selected parts of the study, as well as the fact that the selection of the exposed workers was not described in detail, detract from this otherwise interesting study.

Szulc-Kuberska et al. (1976) studied 50 Polish workers (28 men and 22 women, 25 to 50 years old) with between one and 23 years of occupational exposure to TCE. Of these workers, 44% (22) complained of excessive somnolence, 18% (9) of headaches and 20% of drowsiness during work time. Two instances of loss of consciousness at work were reported. Thirteen workers (26%) reported intolerance to alcohol; in 14 persons (28%), signs of vegetative dystonia (excessive sweating) were present. Four men reported impotency, and three women reported disorders of menstruation, including one with signs of menopause before age 35. Disturbances of affection, such as apathy and inclination to weeping, were also observed. One person revealed signs of psycho-organic syndrome, with disturbances of memory, loss of interest and bradyphrenia. A distinct correlation was found between the duration of work and the frequency of occurrence of symptoms in these workers.

Szulc-Kuberska et al. (1976) also examined the auditory and vestibular apparatus in 40 workers and reported perceptive hearing impairments in 60% of TCE-exposed workers. Workers with previous or present exposure to noise were excluded from this portion of the study. Hearing disturbances were always bilateral and symmetric in the high frequencies, beginnning from 2,000 to 3,000 Hz. Hearing loss was not always correlated with vestibular pathology. Impairment of auditory and labyrinthine function was found more frequently among the workers with longest work histories. These authors stated that disorders of hearing and vestibular reactions were early signs of the adverse health status of workers exposed to TCE.

In all of these workers, the trichloroacetic acid level in the urine exceeded 40 mg/L. No control group was examined, and the type of work and circumstances in the workplace were not described. Air concentrations of TCE in the workplace(s) also were not given. The possible confounding effect of age and length of employment on hearing loss was not discussed. For these reasons, it is difficult to evaluate the results of this study or to determine whether different results would be obtained in a similar but unexposed (to TCE) industrial population.

Axelson et al. (1978) examined the causes of death in a small cohort of 518 men whose TCE exposure was estimated through TCA in the urine. An average TCA level in the urine above 100 mg/L was considered to be high exposure, corresponding to more than 30 ppm in air. Close agreement was found between observed and expected numbers of cancer deaths based on national Swedish cause- and age-specific death rates. The high- and low-exposure groups, comprised 548 and 3,643 person-years of observation, respectively. Due to the small sample size, the cancer risk to man from TCE, particularly with regard to uncommon malignancies, could not be ruled out by these investigators.

All of the epidemiologic studies described above examined workers exposed to TCE in the workplace. A frequent criticism of these studies is that they have rarely included an unexposed group for comparison. The age and sex distribution and other demographic characteristics of the groups of workers being examined were not always provided. Exposed groups were lumped together by intensity of exposure, making it difficult to separate out effects which may hvae been related to age, sex or length of employment. Discussion of exposure to other chemical substances in the workplace and of their possible influence on the findings was also scanty, making the lack of control groups a greater deficiency. In view of these difficulties, information can best be derived by examining consistent findings among studies conducted under different circumstances. Four out of the six studies noted some dose-effect relationship. All but one were able to document exposure to TCE by measuring TCA in the urine of workers. The most consistent findings were complaints of fatigue, alcohol intolerance, disturbances of sleep (both sleepiness and insomnia), headache, dizziness, excess sweating, tachycardia or palpitations and visual disturbances. It should be noted that the studies may not have used comparable methods of ascertaining these symptoms. Some of the similarities between findings may have been related to historical experience of previous investigators and the particular objective of each study.

D. Synergistic and/or Antagonistic Response

Intolerance to alcohol ahs been reported among TCE-exposed workers. Stewart et al. (1974) performed experiments to substantiate this observation. They gave small oral doses of ethanol to seven subjects and exposed them to 20, 100 and 200 ppm of TCE for one, three or seven and a half hours. Transient vasodilation of the superficial skin vessels that reached maximum intensity at 30 minutes was noted.

E. Summary and Conslusions

Reports are available on the accidental ingestion of TCE. A single oral dose of 7.6 g in a four and a half-year-old child produced toxic effects. Assuming a body weight of 20 kg, the estimated dose was approximately 380 mg/kg. In another incident, an adult who ingested 21 g TCE exhibited symptoms such as vomiting, abdominal pain, inebriation, transient unconsciousness and myocardial infarction. In the second case, the dose was estimated to be 300 mg/kg. Therefore, the lowest toxic dose in humans was determined to be 300 to 380 mg/kg.

Occupational exposures have provided some information with regard to exposure and overt adverse health effects. These data, however, do not provide precise exposure levels and are cofounded by the fact that the workers were also exposed concurrently to other chemicals, thus, it was not possible to associate adverse health effects to the chemical(s) with certainty. When the exposure in an electroplating plant was between 627 and 2,093 mg/m^3 for two to three weeks, the workers complained of headaches, muscle and joint pains, nausea, vomiting, loss of appetite, depression, dizziness and narcosis. The workers manifested liver damage as indicated by CCF and hyperglobinemia.

Epidemiological evidence cannot be related to TCE exposure levels with confidence; however, the association between exposure of workers to TCE and observed health effects, including fatigue, dizziness, alcohol intolerance, conduction disturbance of heart muscle, nervous system disorders, increase in plasma Y-globulin and decrease in albumin concentration, is worth mentioning. Some workers were demonstrated to have albumin and elevated urobilinogen in their urine. These studies, however, cannot be used for determining recommended maximum contaminant levels. In addition, intolerance to ethanol has been reported among TCE-exposed workers.

VII. MECHANISMS OF TOXICITY

Exposure to TCE has been reported to produce disturbances in the central nervous system, arrhythmia (cardiotoxic effect), hepatotoxic and nephrotoxic effects and carcinogenic response in animals. Very little is known about the mechanisms by which TCE exerts these bioeffects; however, several attempts have been made to elucidate the mechanisms for some of these bioeffects.

Information concerning the hepatotoxic and possibly the potential carcinogenic effects of TCE has been generated by the experiments of several workers. The first step in this mechanism appears to involve epoxidation of TCE in the mammalian system. This system requires cytochrome P-450 and the NADPH-generating enzymes. The TCE epoxide thus formed may interact: (1) with low molecular weight nucleophiles by conjugation reaction; (2) with cellular macromolecules by alkylation and (3) with water to produce diols or undergo intramolecular rearrangement.

The evidence for macromolecule binding of TCE has been generated by Allemand et al. (1978), Uehleke and Poplawski-Tabarelli (1977), Van Duuren and Banerjee (1976) and Bolt and Filser (1977). In in vitro experiments, Allemand et al. (1978) incubated ^{14}C TCE with rat liver microsomes, with and without the NADPH-generating system. Without the NADPH-generating system, there was negligible radioactivity bound to microsomal proteins. This suggests that TCE itself does not bind to proteins. The TCE-binding was increased after pretreatment with microsomal enzyme inducers and decreased under the influence of CO/O_2 atmosphere and piperonyl butoxide, the inhibitor of microsomal enzymes. Intraperitoneal administration of 100 umol (13.14 mg/kg) of TCE to normal and phenobarbital-pretreated animals produced higher activity in the treated animal tissues. The radioactive material bound to hepatic proteins was 40 times greater than the radioactive material bound to muscle proteins. Inhalation exposure of male Wistar rats to ^{14}C-TCE for five hours at concentrations of 9, 100 and 1,000 ppm (49, 545 and 5,450 mg/m^3) demonstrated that irreversibly bound radioactivity was maximum to the liver and minimum to the muscle (Bolt and Filser 1977). These authors also carried out in vitro covalent-binding experiments utilizing ^{14}C-TCE. Incubating ^{14}C-TCE with NADPH-generating liver microsomes and albumins and globulins, Bolt and Filser (1977) found large amounts of radioactivity bound to albumin (bovine and rabbit). Binding was reduced by the addition of glutathione. This was in contrast to vinyl chloride, where the metabolites preferentially bind to SH groups.

Van Duuren and Banerjee (1976) incubated rat liver microsomes with ^{14}C-TCE. The results showed that TCE binds covalently to microsomal protein. The binding was decreased by the addition of microsomal inhibitors (7,8-benzoflavone), blocked by compound SKF-525A and enhanced by pretreatment of the animals with phenobarbital. Experiments with 3,3,3-trichloropropene oxide (TCPO), a potent inhibitor of epoxide hydrase, showed that this agent causes an enhancement of TCE-binding to microsomal proteins. These results suggest that the binding is via an epoxide or other related electrophilic species. Similar results have been obtained by Uehleke and Poplawski-Tabarelli (1977). Mice were injected intraperitoneally with a solution of ^{14}C-labeled TCE. Microsomes contained the highest amount of irreversibly bound radioactivity. The concentration declined after six hours.

The information cited above suggests that the metabolites of TCE covalently bind with microsomal proteins and that the binding can be increased or decreased by utilizing the enzyme inducers or inhibitors. Covalent protein-binding of metabolites of xenobiotics has been used as a tool to detect whether reactive and possibly hazardous metabolites are formed. Interaction with nucleic acids moieties still has to be examined.

DiRenzo and his co-workers (1982) studied _in vitro_ covalent binding of a series of ^{14}C-labeled aliphatic halides to calf thymus DNA following bioactivation by hepatic microsomes isolated from phenobarbital-treated rats. Six compounds (1,2,-dibromoethane, bromotrichloromethane, trichloroethylene, carbon tetrachloride, chloroform and

1,1,2-trichloroethane) were incubated for 60 minutes (the time period previously determined to produce maximal covalent binding). Halides to DNA adducts were isolated utilizing Sephadex LH-20 column chromatography. Table VII-1 presents the comparative binding to DNA of the selected aliphatic halides. It is noteworthy that the compounds containing bromine were readily bioactivated and bound to DNA to a greater extent than the related chlorine-containing compounds in this series. This is illustrated by the binding to DNA of 1,2-dibromoethane (0.52 ± 0.14) and 1,2-dichloroethane (0.06 ± 0.02). Those aliphatic halides that had the highest levels of covalent binding were those most frequently shown to be carcinogenic in laboratory animals.

Banerjee and Van Duuren (1978) carried out studies on the *in vitro* covalent binding of TCE to salmon sperm DNA in the presence of a microsomal preparation from $B_6C_3F_1$ hybrid mice. The TCE metabolite-DNA adduct was purified by precipitation/reprecipitation technique with solvents and checked for protein and RNA contamination. The TCE-DNA binding was dependent on the concentration of microsomal protein. The amount of binding of TCE to DNA in the presence of microsomes from male mice was higher than that with microsomes from female mice. This correlates with the NCI cancer bioassay on TCE. The binding to DNA was enhanced by *in vivo* pretreatment of the animals with phenobarbital.

Table VII-1 Microsomal Bioactivation and Covalent Binding of Aliphatic Halides to Calf Thymus DNA

Aliphatic halides[a]	Binding to DNA[b]
1,2-Dibromoethane	0.52 ± 0.14 (6)
Bromotrichloromethane	0.51 ± 0.18 (6)
Chloroform	0.46 ± 0.13 (6)
Carbon tetrachloride	0.39 ± 0.08 (6)
Trichloroethylene	0.36 ± 0.14 (7)
1,1,2-Trichloroethane	0.35 ± 0.07 (7)
Dichloromethane	0.11 ± 0.05 (5)
Halothane	0.08 ± 0.01 (6)
1,2-Dichloroethane	0.06 ± 0.02 (6)
1,1,1-Trichloroethane	0.05 ± 0.01 (3)

[a] ^{14}C-labeled aliphatic halides (1 mM) were incubated with hepatic microsomes. Carbon tetrachloride, bromotrichloromethane and halothane were incubated under an N_2 atmosphere, all other incubations were under an O_2 atmosphere, for reasons stated in "Materials and Methods".

[b] In nmol bound/mg DNA/hour. Value are the mean ± standard deviation for the number of experiments in parentheses.

From DiRenzo et al. (1982).

Research on in vivo TCE-DNA binding was performed by Stott et al. 1982. Male $B_6C_3F_1$ mice were dosed by gavage with 1,200 mg/kg ^{14}C-TCE in corn oil. The animals were sacrificed five hours later by decapitation. Livers were frozen and processed for DNA isolation and purification. In three out of four animals, the maximum estimate of the average DNA level was 0.62 ± 0.42 alkylations/10^6 nucleotides. The authors suggested an epigenetic mechanism of tumor formation in the $B_6C_3F_1$ mouse because of the so-called low maximum estimation of alkylation. This conclusion appears to be rather far reaching based on a single experiment in which only a single dose was given and only four animals were used. In addition, the estimate of alkylation with higher standard deviation does not instill confidence in the mind of the reviewer.

In order to establish a correlation between covalent binding and hepatotoxicity, Allemand et al. (1978) demonstrated that intraperitoneal administration of TCE (1,460 mg/kg) to rats resulted in raised SGPT levels, without detectable histologic lesion of the liver. Phenobarbital pretreatment of the animals increased hepatic cytochrome P-450, in vitro formation of the chemically reactive metabolite of TCE, the amount of metabolite bound in vivo and the hepatotoxicity of a 1,460-mg/kg dose of TCE. The inhibition of TCE metabolism with $CoCl_2$ decreased the hepatic cytochrome P-450, the in vitro formation rate of the chemically reactive metabolite of TCE and the hepatotoxicity of a 1-mL/kg dose of TCE. In an inhalation experiment, Carlson (1974) observed enhancement of TCE hepatotoxicity in male rats pretreated with phenobarbital and 3-methylcholanthrene. Indices of hepatotoxicity were serum isocitrate dehydrogenase, SGPT, SGOT and hepatic glucose-6-phosphatase; TCE exposure levels ranged from 10,400 to 6,900 ppm, and exposure lasted for two hours. Moslen et al. (1977b) exposed male rats, after pretreatment with five different inducers of hepatic mixed-function oxidases, to 1% (10,000 ppm) TCE for two hours. The magnitude of induction of cytochrome P-450 correlated with the extent of TCE-induced liver injury, as measured by serum transaminase levels ($r = 0.95$), with prolongation of anesthesia recovery time ($r = 0.95$) and with enhanced urinary excretion of trichlorinated metabolites ($r = 0.88$).

Factors other than enzyme induction, including changes in the redox state of the hepatocytes or depletion of co-factors required for specific metabolic steps, could also influence the hepatotoxicity of TCE. Cornish and Adefuin (1966) reported increased hepatotoxicity in rats pretreated with ethanol. An explanation for the observed interaction of ethanol and TCE is the availability of NAD and NADPH, the co-factors required for the metabolism of TCE, at the step involving biotransformation of chloral hydrate. The concentration of glutathione, an endogenous compound responsible for varied types of metabolic reactions in mammalian systems, has been demonstrated to be affected by the administration of TCE to the animals.

After TCE administration to normal rats, hepatic glutathione was decreased. This was not true when the animals were pretreated with the chemicals which inhibit metabolism, suggesting that glutathione depletion was related to TCE metabolism. In vitro addition of glutathione to the incubation mixture decreased the amount of TCE metabolite bound to microsomal proteins. As it was reported earlier, tissue binding of TCE metabolite has been related to hepatotoxicity.

Salvolainen (1977) reviewed some aspects of the mechanisms by which industrial solvents produce neurotoxic effects. Neurotoxic actions may be described as responses that are related to nervous system function and/or structure. The acute effects appear to be derived from the direct action of solvents on nerve cell membranes, whereas the development of chronic effect depends more on the metabolic effects of the individual chemical. The elicitation of anesthesia in surgical operations may be considered an example of the former effect. The majority of such effects are probably reversible. It appears that when it is used for anesthesia, TCE would fall in the category of chemicals which interfere with the nerve cell membrane. In regard to the chronic effects, metabolic changes have been cited for the neurotoxic effects of many chemicals. Specific effects on neuronal metabolisms and functions due to exposure to TCE have not been examined.

Evidence has been generated which demonstrates that it is the metabolite of TCE, rather TCE itself, which is responsible for hepatotoxic and potential carcinogenic responses. Experiments have suggested that metabolite of TCE covalently binds with the macromolecules, including DNA. It appears that TCE, when used for its anesthetic response, may fall in the category of chemicals which interfere with the nerve cell membrane.

VIII. RISK ASSESSMENT

The National Academy of Sciences (NAS 1977) has made an assessment of the human cancer risk associated with TCE in drinking water. This risk assessment was based upon the results of a carcinogenesis bioassay experiment with animals (NCI 1976). In this study, highly significant differences in the incidence of hepatocellular carcinomas were found between treated and control mice of both sexes.

The available sets of dose-response data were individually considered according to the risk section in the chapter on margin of safety. Each set of dose-response data was used to statistically estimate both the lifetime risk and an upper 95% confidence bound on the lifetime risk at the low-dose level. These estimates are of lifetime human risks and have been corrected for species conversion on the dose/surface area basis. The risk estimates are expressed as a probability of cancer after a lifetime consumption of 1 L of water/day containing Q ppb of the compound of interest. For example, a risk of 1×10^{-6} Q implies a lifetime probability of cancer of 2×10^{-5} if 2 L/day were consumed and the concentration of the carcinogen was 10 ppb (i.e., Q = 10). This means that at a concentration of 10 ppb during a lifetime of exposure, this compound would be expected to produce one excess case of cancer for every 50,000 persons exposed. If the population of the United States is taken to be 220 million, this translates into 4,400 excess lifetime deaths from cancer, or 62.8/year. Since several data sets are typically available, the range of the low-dose risk estimates are reported. For TCE at a concentration of 1 ug/L (Q = 1), the estimated risk for man would be 0.36 to 1.1×10^{-7} Q. The upper 95% confidence estimate of risk at the same concentration is 0.55 to 1.6×10^{-7}.

It should be emphasized that these extrapolations are based on a number of unverifiable assumptions: (1) extrapolation from high exposure to low exposure in mice on the basis of a multi-stage mathematical model; (2) extrapolation from mouse to man, on the basis of the surface-area rule and 3) extrapolation from gavage exposure to oral exposure assumed equal. These estimated human risks should be taken as crude estimates at best.

Using an "improved" multi-stage model, the CAG has determined that 27 ug/L at 2 L/day over a lifetime would result in an excess cancer risk estimate of 10^{-5} at the 95% confidence limit.

The NAS and EPA's CAG have calculaged the projected incremental excess cancer risks associated with consumption of a specific chemical via drinking water by mathematical extrapolation from high-dose animal studies. Using the risk estimates generated by the NAS (1977-1979), in which the

the multi-stage model was utilized, the range of TCE concentrations was computed that would nominally increase the risk of one excess cancer per million (10^6), per hundred thousand (10^5) or per ten thousand (10^4) people over a 70-year lifetime, assuming daily consumption at the stated exposure level. From the NAS model, it was estimated at the 95% confidence limit that, over a lifetime, consuming 2 L of water/day having a TCE concentration of 450 ug/L, 45 ug/L or 4.5 ug/L would increase the risk of one excess cancer per 10^4, 10^5 or 10^6 people exposed, respectively. Using the revised CAG approach, and thus the "improved" multi-stage model, it can be estimated at the 95% confidence limit that, over a lifetime, consuming 2 L of water/day having a TCE concentration of 280 ug/L, 28 ug/L or 2.8 ug/L would increase the risk of one excess cancer per 10^4, 10^5 or 10^6 people exposed, respectively.

The numerical differences observed after utilizing the NAS and the CAG risk estimates (see Table VIII-1) are partly due to the fact that the dose extrapolation models used by the two groups are similar, but not identical. The NAS has used the multi-stage model, whereas the CAG has used the "improved" version of the multi-stage model which was recently discussed by Crump (USEPA 1980). In addition, the selection of the data and other parameters in each model also result in some differences in the risk assessments.

Table VIII-1 Drinking Water Concentrations and Associated Cancer Risks

Excess lifetime cancer risk	Range of concentrations (ug/L)[a]		
	CAG (95% confidence limit)	NAS (95% confidence limit)	NAS (point estimate)
10^{-4}	280	450	1,400-450
10^{-5}	28	45	140-45
10^{-6}	2.8	4.5	14-4.5

[a] Assuming 2 L of water are consumed per day.

IX. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Adjusted Acceptable Daily Intake (ADI) for the chemical is determined. For ingestion data, this approach is illustrated as follows:

$$\text{Adjusted ADI} = \frac{(\text{NOAEL or MEL in mg/kg})(70\text{ kg})}{(\text{Uncertainty Factor})(2\text{ L/day})}$$

The 70-kg adult consuming 2 L of water per day is used as the basis for the calculations. A "No-Observed-Adverse-Effect Level" (NOAEL) or a "minimal-effect level" (MEL) is determined from animal toxicity data or human effects data. This level is divided by an uncertainty factor because, for those numbers which are derived from animal studies, there is no universally acceptable quantitative method to extrapolate from animals to humans, and the possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human toxicity data, an uncertainty factor is used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxins. The guidelines set forth by the NAS (1977) are used in establishing uncertainty factors. These guidelines are as follows: an uncertainty factor of 10 is used if there exist valid experimental results on ingestion by humans, an uncertainty factor of 100 is used if there exist valid results on long-term feeding studies on experimental animals, and an uncertainty factor of 1,000 is used if only limited data are available.

In the quantification of carcinogenic effects, mathematical models are used to calculate the estimated excess cancer risks associated with the consumption of a chemical through the drinking water. The EPA's CAG has used the multi-stage model, which is linear at low doses and does not exhibit a threshold, to extrapolate from high-dose animal studies to the low doses of the chemical expected in the environment. This model estimates the upper bound (95% confidence limit) of the incremental excess cancer rate that would be projected at a specific exposure level for a 70-kg adult, consuming 2 L of water/day, over a 70-year life span. Excess cancer risk rates also can be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. Current understanding of the biological mechanisms involved in cancer do not allow for choosing among the models. The estimates of incremental risks associated with exposure to low doses of potential carcinogens can differ by several orders of magnitude when these models are applied. The linear, non-threshold multi-stage model often gives one of the highest risk estimates per dose and thus would usually be the one most consistent with a regulatory philosophy which would avoid underestimating potential risk.

The scientific data base, which is used to support the estimating of risk rate levels as well as other scientific endeavors, has an inherent uncertainty. In addition, in many areas, there exists only limited knowledge concerning the health effects of contaminants at levels found in drinking water. Thus, the dose-response data gathered at high levels of exposure are used for extrapolation to estimate responses at levels of exposure nearer to the range in which a standard might be set. In most cases, data exist only for animals, and uncertainty exists when the data are extrapolated to humans. When estimating risk rate levels, several other areas of uncertainty exist, such as the effect of age, sex, species and target organ of the test animals used in the experiment and the exposure mode and dosing rates. Additional uncertainty exists when there is exposure to more than one contaminant due to the lack of information about possible additive, synergistic or antagonistic interactions.

Trichloroethylene studies which provide doses versus adverse health effects in humans are not available. Therefore, estimations based upon the best scientific judgments in experimental animals are required to quantify toxicological effects (QTE) with respect to concentrations in drinking water. With this objective in mind, this section analyzes the data, taking into consideration interspecies variation, observed adverse health effects (both carcinogenic and noncarcinogenic) and dosages.

A. Non-Carcinogenic Effects

Among the acute and chronic adverse effects due to TCE exposure, hepatotoxicity appears to be of most significance. All the animal species which have been studied have responded to the hepatotoxic effects of TCE, with the intensity of the response being dependent upon the dose and the duration of exposure. There have been reports concerning the nephrotoxic effects of TCE, and central nervous system and cardiotoxic effects have been observed at very high concentrations.

Several inhalation studies have provided observations on hepatotoxic effects after single or multiple exposures. Kylin et al. (1965) compared the hepatotoxicity of chloroform, TCE and tetrachloroethylene. Mice were given TCE by inhalation for a single four-hour time period. The animals were sacrificed on the third day; the livers were analyzed by histological examination and by acetone-hexane extraction for fat. In addition, activity of serum ornithine carbamyl transferase was determined. At a concentration level of 6,400 ppm, TCE produced no significant damage to the liver. In this study, TCE was the least hepatotoxic chemical, whereas chloroform was the most hepatotoxic. Similar results were obtained by Plaa et al. (1958) and Gehring (1968) when animals were exposed to halogenated hydrocarbon solvents by subcutaneous injection and inhalation. The results of these workers indicated that the halogenated hydrocarbon solvents, in the order of their decreasing capacity to cause liver dysfunction rank: carbon tetrachloride, chloroform, 1,1,2-trichloroethane, tetrachloroethylene, TCE 1,1,1-trichloroethane.

Multiple inhalation exposure studies have been reported utilizing mice, rats and dogs. Seifter (1944) observed degeneration of liver parenchyma cells in dogs that were exposed to either 750 ppm TCE for 8 hours/day, 6 days/week, for 3 weeks, or 500 to 750 ppm TCE for 6 hours/day, 5 days/week, for 8 weeks. Slight fatty infiltration of the liver of mice was detected by Kylin et al. (1965). These workers exposed female albino mice to 1,600 ppm TCE by inhalation for four hours daily, six days a week, over periods of one, two, four and eight weeks. The increase in liver fat content was detectable after one week of exposure; subsequently, the liver fat showed no further increase. In terms of fatty degeneration of the liver, the authors noticed that tetrachloroethylene was approximately 1/10 times less toxic than trichloroethylene. Male Wistar II rats that inhaled 55 ppm TCE for 14 weeks exhibited elevated liver weights, but did not manifest pathological changes as measured by histopathological examinations, liver function tests, renal function tests and blood glucose (Kimmerle and Eben 1973a). Four animal species (rabbits, guinea pigs, rats and monkeys) were exposed to 100 to 3,000 ppm TCE vapor for seven hours daily, five days a week, for approximately six months by Adams et al. (1951). Rats exposed to 300 to 3,000 ppm TCE for a period of 36 days (total of 27 exposures) showed an increase in liver and kidney weights. Histopathological examination of the tissues, however, failed to reveal any abnormality in male rats, although some female rats showed fat vacuoles in the cytoplasm of the liver. Rats exposed to 200 ppm TCE for 205 days (151 total exposures) showed no significant abnormality compared to the controls. The authors concluded that the maximum concentrations without adverse effects were as follows: monkey, 400 ppm; rat and rabbit, 200 ppm; guinea pig, 100 ppm.

Because of its effects on the nervous system, TCE has been used as a general anesthetic agent. Studies performed as early as 1944 give information concerning the blood concentrations of TCE necessary for lethal as well as anesthetic effects. Dogs, rabbits, guinea pigs and cats were administered TCE by inhalation, and blood levels were determined at death and anesthesia stages. The lethal blood TCE concentration in dogs was found to be from 100 to 110 mg/100 mL blood. At the anesthetic stage, TCE blood levels were 24 to 37, 23 to 28, 14 to 18 and 25 to 32 mg/100 mL blood for dogs, rabbits, guinea pigs and cats, respectively. As with the liver, guinea pigs appeared to be the most sensitive species among the experimental animals studied with respect to the anesthetic response. The blood-brain ratio at anesthetic dosages was approximately 1:2 for both guinea pigs and dogs (Kulkarni 1944).

Histopathological changes in neural tissues have been observed on acute and long-term exposure of animals to TCE. A single exposure of dogs to 30,000 ppm TCE in air resulted in death within 20 minutes. No obvious changes were found in the nervous system. In a more long-term experiment, the animals were subjected to TCE concentrations ranging from 500 to 3,000 ppm for periods varying from two to eight hours/day, often for five days/week. The total exposure period was between 60 and 162 hours. The exposures appeared to selectively destroy the Purkinje layer of the cerebellum. The cerebral hemispheres showed mild changes; scattered cortical neurons became swollen or pyknotic, and the white matter of the myelin developed a mild focal swelling (Baker 1958). Bartonicek and Brun (1970) injected TCE intramuscularly in female rabbits and observed moderate neurological changes in the exposed animals. The dosage regimen included subacute exposure for 29 days to injections of 2.47 g/kg body weight three times a week. For the chronic exposure experiment, the animals were injected intramuscularly with 1.62 g/kg body weight twice a week for 41 to 247 days. The rabbits were sacrificed at different times during the test, and the brains were examined histologically and histochemically for any pathological change. Round cell infiltration around blood vessels and in the parenchyma occurred in all animals in the subacute test and in one of the animals in the chronic experiment, but not in the controls. Disappearance of Purkinje cells and basket cells was definitely shown only in the chronic experiment.

Grandjean (1960) exposed male rats to 200 and 800 ppm TCE vapors for 4 to 11 weeks. The rats were subjected to a single three-hour TCE exposure just before testing. After the exposure, trained rats, responding to signals, climbed up a rope to reach a feeding trough in which they found a small dextrose pellet as a reward. The results indicated that the increase in the number of spontaneous climbs after exposure to the solvent was significant in comparison with the control tests. The observed effect was not dose-dependent. The author concluded that TCE, in the doses studied, modified the psychological equilibrium of rats by increasing excitability. In a 1963 report, the author (Grandjean 1963) described the effect of TCE vapors on the swimming performance and motor activity of rats. The animals were exposed for six hours, and swimming tests were performed 5 to 15 minutes later. At 400 ppm, TCE retarded the performance only of those rats swimming with an additional load (in a manner barely significant), while 800 ppm adversely affected the performance, both with and without the load, in a significant manner. One hour after termination of exposure, no significant changes in the swimming times were observed.

Reports on the accidental ingestion of TCE are available. A single oral dose of 7.6 g in a four and a half-year-old child produced toxic effects. Assuming a body weight of 20 kg, the estimated dose was approximately 380 mg/kg. In another incident, an adult who ingested 21 g of TCE exhibited symptoms such as vomiting, abdominal pain, inebriation, transient unconsciousness and myocardial infarction. In the second case, the dose was estimated at 300 mg/kg. Therefore, the lowest toxic dose in humans taken to be 300 to 380 mg/kg.

Occupational exposures have given some information with regard to exposure and overt adverse health effects. The data, however, did not provide precise exposure levels and were confounded by the fact that the workers were also exposed concurrently to other chemicals. It was not possible to associate adverse health effects to the chemical(s) with certainty. In an electroplating plant, when the exposure was between 627 and 2,093 mg/m^3 for two to three weeks, the workers complained of headaches, muscle and joint pains, nausea, vomiting, loss of appetite, depression, dizziness and narcosis. the workers exhibited liver damage as indicated by CCF and hyperglobinemia.

Epidemiological evidence cannot be related to the exposure levels with confidence; however, the association between TCE exposure and observed health effects, such as fatigue, dizziness, alcohol intolerance, conduction disturbance of heart muscle, nervous system disorders, increase in plasma Y-globulin and decrease in albumin concentration, is worth mentioning.

Some workers had albumin and elevated urobilinogen in their urine. These studies cannot be used for determining the quantification of toxicological effects (QTE).

B. Quantification of Non-Carcinogenic Effects

Similar bioeffects, across species (humans, dogs rabbits, guinea pigs, rats and mice), as a result of TCE exposure by either inhalation, intramuscular injection or gavage have been reported. Trichloroethylene has been demonstrated to have effects on the central nervous system, liver and heart. It has also been shown to be carcinogenic in mice in two studies. Because of the special nature of the carcinogenic effect, it is discussed separately in this section.

The central nervous system and the liver of the mammalian system appear to be the sensitive end points with respect to adverse health effects. There are limited data concerning dosage, duration of exposure and effects on the central nervous system of TCE. There is only one study in which human volunteers were exposed to 600 mg/m^3 TCE for two 4-hour periods. In this study, psychophysiological changes were noted in human volunteers. This study cannot be used for recommending a more long-term exposure or QTE.

Liver toxicity should be used as an end point, for estimating QTE for TCE in drinking water. Trichloroethylene has been shown to damage the liver of humans as indicated by CCF and hyperglobinemia. The exposure related to this effect was between 627 and 2,093 mg/m^3 for two to three weeks. The exposure (in mg/kg/day) can be estimated as:

$$\frac{627 \times 10 \times 0.3}{70} = 26.87 \text{ mg/kg}$$

where:

627 = lowest estimated exposure dose in mg/m^3.

10 = m^3 of air-TCE mixture inhaled.

0.3 = assumed fraction of TCE retained in the body after inhalation.

70 = average body weight of an adult human in kg.

Rats exposed to 300 mg/m^3 (55 ppm) for five days/week for 14 weeks had elevated liver weights. Assuming the lung-whole body weight ratios for humans (adults) and rats (adults) to be roughly equivalent, the total dose of TCE to humans can be estimated. The calculations are:

$$\frac{(300 \text{ mg/m}^3)\ 8 \text{ m}^3\text{/day}\ (5)\ (0.30)}{(7)} = 514 \text{ mg/day}$$

where:

55 ppm = 300 mg/m^3=minimum effect level.

8 m^3 = air inhaled during the experiment.

5/7 = fraction converting from 5- to 7-day exposure.

0.30 = absorption rate.

Dosages which adversely affect the liver of humans are thus estimated to be from 26.87 to 89.7 mg/kg for an exposure period of two to three weeks. Two to three weeks is too short an exposure period to estimate an ADI for humans. Furthermore, this was a very crude estimate, and the studies were not well controlled.

The rat study estimate of 7.34 mg/kg (513.8 mg for a 70-kg adult) as an adverse-health-effect dose appears to be a reasonable level for the calculation of an ADI. If the 7.34-mg/kg dose is accepted as a minimum-effect dose, an uncertainty factor of 1,000 can be applied to calculate an ADI. The calculation is:

$$\text{ADI} = \frac{7.34\ \text{mg/kg} \times 70\ \text{kg}}{1{,}000} = 0.514\ \text{mg/day}$$

The ADI of TCE, using non-carcinogenic data and assuming 100% exposure from drinking water, is 0.514 mg/day. It should be appropriately reduced if there is also TCE exposure from other sources, such as food and air. If 100% exposure from drinking water is assumed, then an adjusted ADI can be calculated as:

$$\text{Adjusted ADI} = \frac{0.514\ \text{mg}}{2\ \text{L}} = 0.257\ \text{mg/L}$$

C. Carcinogenic Effects

Bacterial mutagenesis sytems are most commonly used as a screening technique to determine the mutagenic and carcinogenic potential of chemicals. Trichloroethylene was found to be mutagenic in *Salmonella typhimurium* strains and the *E. coli* K 12 strain utilizing liver microsomes for activation (Greim et al. 1975, 1977). Bartsch et al. (1979) used S-9 fractions from liver specimens instead of microsomes for activation in the mutagenesis test. The authors reported that TCE was marginally mutagenic. Waskell (1978) reported that TCE was non-mutagenic in the Ames test system with activation. The negative responses obtained by later research cannot be explained at the present time.

Saccharomyces cerevisiae (yeast) and Fisher rat embryo have also been used to study mutagenic response. After activation with liver microsomal fractions, TCE was mutagenic in Sacchromyces cerevisiae strains D_4, D_7 and XV185-14C (Bronzetti et al. 1978, Shahin and vonBorstel 1977). Price et al. (1978) tested TCE for in vitro cell-transforming potential in a Fisher rat embryo system (F1706). The transformed cells grew in a semi-solid agar and produced undifferentiated fibrosarcomas when inoculated into newborn Fisher rats.

The National Cancer Institute (NCI 1976) reported that TCE induced cancer in mice. Trichloroethylene was administered by oral gavage, five times per week for 78 weeks. The time-weighted average daily doses were 1,169 and 2,339 mg kg for male mice and 869 and 1,739 mg/kg for female mice. These tests were conducted using industrial grade (99% pure) TCE on Osborne-Mendel rats and $B_6C_3F_1$ mice. A complete necropsy and microscopic evaluation were conducted on all the animals (except seven out of the original 480) which died at unscheduled times.

No significant difference was noted in neoplasms between experimental and control groups of rats. In both male and female mice, however, the higher dose induced primary malignant tumors in the liver. For males, 26 of 50 mice receiving the low dosage and 31 of 48 mice receiving the high dosage developed hepatocellular carcinomas, while only one of 20 controls showed neoplasms. In female mice, 4 of 50 mice receiving the low dosage and 11 of 47 mice receiving the high dosage developed neoplasms, as compared to zero out of 20 controls.

In the NCI study cited above, the TCE used in the test was later found to contain epichlorohydrin, a carcinogen. Therefore, NCI repeated the bioassay with epichlorohydrin-free TCE. Rats (F344/N) and mice ($B_6C_3F_1$) of both sexes were used. Trichloroethylene was mixed with corn oil and administered by gavage five times per week for 103 weeks. Rats received dosages of 500 and 1,000 mg/kg. These dose levels were lower than the initial doses used in the earlier bioassay in Osborne-Mendel rats (650 and 1,300 mg/kg for both sexes). The dosage levels used in the mice were also lower than in the earlier study. The dose selected for the study in mice was 1,000 mg/kg for both sexes.

Trichloroethylene was not found to be carcinogenic in female F344/N rats. The experiment with male rats was considered to be inadequate, since these rats received dose levels of TCE which exceeded the maximum tolerated dose. Trichloroethylene was carcinogenic in both sexes of $B_6C_3F_1$ mice, producing hepatocellular carcinomas in males and females.

D. Quantification of Carcinogenic Effects

To assist the regulators in making decisions for the control of chemical carcinogens in the environment, several scientists have attempted to estimate the excess cancer risk due to exposure to carcinogens. With respect to contamination of water with carcinogens, the NAS and EPA's CAG have calculated additional cancer risk estimates.

Using the revised CAG approach, and thus the "improved" multi-stage model, it can be estimated that water with TCE concentrations of 280 ug/L, 28 ug/L or 2.8 ug/L would increase the risk of one excess cancer per 10^4, 10^5 or 10^6 people exposed, respectively. These estimates were calculated from the NCI bioassay data, which utilized TCE contaminated with epichlorohydrin. Since then, an NCI bioassay utilizing epichlorohydrin-free TCE has become available; the data from this bioassay have been reviewed and evaluated for carcinogenicity, and epichlorohydrin-free TCE has been reported to be carcinogenic in mice.

E. QTE Development

Several organizations have attempted to derive acceptable levels of TCE in water. These values are given in Table IX-1. The NAS (1977) estimated the excess cancer risk due to human exposure to TCE in drinking water. They used a multi-stage model for their calculations. The cancer risk estimate at the upper 95% confidence level of 1 ug/L TCE was 0.55×10^{-7}. This translated into a concentration of 45 ug/L for a risk of 10^{-5}. The estimate reported by the EPA's Office of Water Regulation and Standards (OWRS) for an identical risk is 27 ug/L. These calculations take into consideration the average amount of fish consumed daily by an individual. The differences in the two estimates may be attributed to the different mathematical models used and the assumptions made for these calculations (such as the consumption of fish by an individual and the animal species used). The World Health Organization (WHO 1981) published a tentative guideline level of 30 ug/L based on the NCI mouse data which utilized a linear multi-stage extrapolation model. It is noteworthy that these risk estimates were made utilizing the total exposure from drinking water. The risk would be proportionally increased if the exposure from air and food was taken into consideration.

Table IX-1 Recommended Concentrations of TCE in Drinking Water

Organization	Non-carcinogenic end point	Carcinogenic end point
NAS (1977)	--	--
NAS (1980)	105 mg/L for 1 day 15 mg/L for 10 days	--
USEPA (OWRS)	6.77 mg/L for lifetime	27 ug/L
USEPA (ODW, HAs)	2 mg/L for 1 day 0.2 mg/L for 10 days 0.080 mg/L for long-term	--
WHO	--	30 ug/L

The NAS (1980) also calculated levels of TCE for a short-term exposure. In these estimations, the carcinogenic potential of TCE was not taken into consideration. Concentrations for one-day and seven-day exposures were estimated to be 105 and 15 mg/L, respectively. These calculations were based on the rough approximation of a toxic dose in an accidental exposure case; it was not a controlled experiment in which the subjects were exposed to several dose levels, and the no-effect dose level was not established. The EPA's OWRS established a level of 6.77 mg/L, estimated assuming a Threshold Limit Value (TLV) of 100 ppm and an average daily consumption of 6.5 g fish by an individual. It is worth mentioning that TLVs are established for healthy adult workers (mostly males) and are not recommended for the general public, in which the population consists of sick as well as healthy individuals of both sexes. The NAS also calculated an alternate level in which a single dose of 2.38 mg/kg/day was used This study was for a short duration and should not be used for estimating a lifetime acceptable level.

The Office of Drinking Water issued a Health Advisory (formerly called a SNARL) in 1977. This Health Advisory estimated one-day, ten-day and long-term negligible risk levels to be 2, 0.2 and 0.080 mg/L, respectively. More data have become available since 1977, and these levels should be evaluated and revised, if necessary. It should be remembered that these health advisories were established for transient exposures; they do not take cancer risk estimates into consideration, and they do not incorporate the exposure of humans to TCE from sources such as food and air.

Use of a two-year feeding study in at least two experimental animals (one of them being a rodent) would be the best means of calculating an ADI. Since such data are not available, an ADI of 0.514 mg/day has been calculated from a three-month inhalation study in rats.

Since it is assumed that humans consume about 2 L of water/day, the adjusted ADI would be:

$$\text{Adjusted ADI} = \frac{0.514\ \text{mg}}{2\ \text{L}} = 0.257\ \text{mg/L}$$

The carcinogenic potential of TCE was not taken into consideration in the above calculation of the ADI; however, this aspect of adverse health effects should not be ignored, although there is limited evidence concerning the carcinogenicity of TCE.

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